THE DISTURBING EFFECT OF THE GLUTINOUS GENE IN RICE ON A MENDELIAN RATIO[1]

LIEN FANG CHAO

University of Wisconsin, Madison, Wisconsin

Received August 10, 1927

TABLE OF CONTENTS

	PAGE
Nature of the glutinous character and its significance	191
Review of literature	192
Material and methods	194
Experiments and results	
Distribution of glutinous and non-glutinous grains in F_2 from reciprocal crosses between two Manchurian varieties	198
Comparison of F_2 distributions in successive crops of 100×200	199
Distribution of F_2 classes from 14 combinations of unrelated varieties	200
Corroborative evidence for the accumulation of deficiencies	202
Cause of the deficiency of glutinous grains	
Accuracy of classification	203
Effect of seasonal infertility	204
Normality of segregation at microsporogenesis	208
Possibility of an accessory factor	209
Evidence from the F_2 generation	210
Evidence from the F_3 generation	213
DISCUSSION	214
SUMMARY	220
LITERATURE CITED	221
APPENDIX	223

NATURE OF THE GLUTINOUS CHARACTER AND ITS SIGNIFICANCE

According to texture of endosperm, rice varieties are popularly divided into main groups, namely, starchy and glutinous. The endosperm of the former is hard and corneous and that of the latter is softer and opaque. When boiled, glutinous rice forms a glue-like mass, while in common starchy rice, when this is properly cooked, the grains remain free and entirely distinct. To this property of forming a sticky mass, the glutinous rice owes its name; but the substance responsible for this behavior is a carbohydrate rather than gluten.

From the genetic standpoint, it has been shown, by several investigators whose work will be reviewed presently, that the glutinous character is

[1] Papers from the Department of Genetics, Agricultural Experiment Station, UNIVERSITY OF WISCONSIN, No. 79. Published with the approval of the Director of the Station.

governed by a simple Mendelian recessive gene. This simple recessive factor exerts its influence not only in the endosperm, but also in the pollen in such a way that the carbohydrate reserve material gives a reddish-brown reaction to the iodine reagent instead of the normal blue reaction (PARNELL 1921).

Since it affects the endosperm, the glutinous character is visible sooner than other sporophytic characters. It is readily studied in large numbers, an average panicle bearing several hundred grains. The character is also manifested in the gametophyte generation, so that direct results of Mendelian segregation with regard to the glutinous pair of factors, *Gl gl*, can be observed. Its further significance lies in the fact that it is very similar to the waxy character in maize, which has recently aroused a good deal of genetic interest.

Fom the practical standpoint, glutinous rice is also very valuable, particularly in the Orient. In China, it is universally used in different forms of pastries on several national celebrations, such as the "Lantern Festival," the "Dragon Festival," and others. The straw of glutinous varieties is more pliable and has greater tensile strength than that of starchy rices, qualities highly valued in certain industries. Both the grain and the straw command a premium on the market. Glutinous rice, like the starchy class, is very rich in varieties. Thus, of the 974 classified Philippine varieties, 144 are glutinous. Corresponding figures for Javanese varieties are 756 and 41, according to COPELAND (1924). While the Occident does not raise the glutinous rice to any extent, about 8 percent of the rice area in Japan is planted to this sort and in China the estimate is as high as 20-30 percent.

REVIEW OF LITERATURE

Mendelian characters which are expressed in the endosperm have been found in barley, rye, wheat, and corn. COLLINS (1909) found, in a variety of maize imported from China, a new type of endosperm later known as waxy. Studies on the inheritance of the waxy character by COLLINS and KEMPTON (1911) and KEMPTON (1919) demonstrated that the waxy character behaves as a simple Mendelian recessive and that a significant deficiency of waxy kernels occurs in F_2 when heterozygous individuals are self-pollinated. That the waxy character in maize is also manifested in the pollen grains is shown by BRINK and MACGILLIVRAY (1924), DEMEREC (1924), and LONGLEY (1924). BRINK (1925) and KIESSELBACH and PETERSEN (1926), further found it manifested in the female gametophyte. To account for the deficiency of the waxy kernels, BRINK and MACGILLIVRAY

(1924) suggested a differential rate of pollen tube growth. MANGELSDORF and JONES (1926) postulated that this differential pollen tube growth is due to the presence of an accessory factor linked with the $W_x w_x$ pair rather than to the waxy gene itself.

The glutinous character in rice is similar to the waxy character in maize. The inheritance of this character has been studied by several investigators.

HOSHINO (1902) observed xenia in his crosses between *Oryza glutinosa* and *O. sativa*.

KATO also studied the glutinous character, but I have been able to secure his data only from a review paper by CHANG (1921). KATO's F_2 population consisted of 286 non-glutinous grains and 101 glutinous ones, whereas 290.25 and 96.75 are expected, respectively, on the basis of a 3:1 ratio. This gave a plus deviation of 4.25 glutinous grains which is, however, only 0.74 times the probable error.

In accord with KATO's data, IKENO (1914) obtained a larger excess of glutinous grains. In a total F_2 population of 7916 grains, there is a plus deviation of 2.11 times the probable error. Further analysis of IKENO's data, however, reveals the fact that the results of his reciprocal crosses stand in contrast to each other, as shown in the following table reconstructed from the original data:

	Non-glutinous grains	*Glutinous grains*	*Total*	*Deviation*	*Dev. / P.E.*
Common × glutinous	637	178	815	−25.75	3.08
Glutinous × common	5245	1856	7101	80.75	3.27
Total	5882	2034	7916	55.0	2.11
Expected	*5937*	*1979*			

Here, common × glutinous gave a deficiency of the glutinous type which is over three times the probable error, and the reverse condition happened in the reciprocal cross.

PARNELL's (1921) data show a significant deficiency in the glutinous class. From nine F_1 plants, each giving about the same ratio, he obtained 5292 non-glutinous grains and 1587 glutinous ones, whereas 5159 and 1720 are the expected values. This gives a deviation of −133 individuals, which is 5.49 times the probable error. Here, then, the deficiency is significant for the odds against the occurrence of such a deviation being due to chance alone are several thousand to one.

YAMAGUCHI (1926) studied two cases. In one of these, the ratio of non-glutinous and glutinous grains in F_2 is 3490:925, showing a minus deviation of the recessive class over 9.21 times the probable error. In the other case, the ratio is 1754:709, giving a plus deviation that is 6.47 times the probable error. He attempted to explain the deficiency in the first case on the basis of two factors, *H* and *I*:

1 *HHII* 2 *HHIi* 2 *HhII* 4 *HhIi* 1 *HHii* 2 *Hhii* 1 *hhII*	13 starchy	2 *hhIi* 1 *hhii*	3 glutinous

Factor *H* prevents hydrolysis of the starch reserve, whereas in the absence of *H*, or presence of *h*, the starch is further hydrolyzed and erythrodextrin (?) results. The factor *I* is similar, but is less active than *H* so that two doses of *I* are necessary in order that the carbohydrate remains as starch. The presence of one dose of *I* and two doses of *i* occasions the hydrolysis of the starch into erythrodextrin (?). This dihybrid hypothesis, however, does not explain the excess of recessives in his second case; furthermore, it meets other serious difficulties, one of which is that the glutinous genotype, *hhIi*, should throw starchy grains in the next generation on self-pollination.

The present investigation was started in the fall of 1924. The interest in this problem is threefold. We have sought (1) to arrive at a definite interpretation of the observed facts in terms of the distribution of genetic factors; (2) to clarify the results obtained by the earlier workers on this subject; and (3) we have attempted to discover when and how the germinal elements involved bring about their differential effect.

MATERIAL AND METHODS

In this study, twelve varieties of glutinous and non-glutinous rice were used.

100 A glutinous variety known as "Chhalbe," *Oryza sativa* L., obtained from the MANCHURIAN AGRICULTURAL EXPERMENTAL STATION, in 1925.

200 A non-glutinous variety known as "Han diouza" obtained from the same source as 100.

300 A selection of a glutinous rice, known as C. I. No. 654-1, obtained from Mr. J. M. JENKINS of the RICE EXPERIMENTAL STATION, Crowley, Louisiana in 1924.

400 A pure line known as Fortuna C. I. No. 1344, selected from a non-glutinous variety "Pa Chiam" of Formosa, obtained from the same source as 300.

600 A non-glutinous variety known as "Yu-Tao" or "Chinese Imperial Rice," obtained from Mr. C. C. CHEN of TSING HUA COLLEGE, Peking, China.

700 A non-glutinous variety known as "Ta peh mon" or "big white awned," obtained from Mr. C. Y. PAN of PEKING AGRICULTURAL UNIVERSITY.

800b A non-glutinous strain isolated from a Chinese variety known as "Hei Lo Tao," native to Shen-si Province, China.

900 A glutinous variety grown in Tseng Hua district, Chihli Province, China, obtained from the same source as 700.

1000 A non-glutinous variety known as "Shang-Tao" or scented rice, grown in Shen-si Province, China.

1300 A glutinous variety known as "Acupangna," obtained from Mr. Higgins of the UNIVERSITY OF THE PHILIPPINES.

Using the varieties listed above as parental stocks, many cross-pollinations were made in different combinations, and 573 successful crosses were obtained. F_2 and F_3 generations were raised from these crosses.

An iodine solution was used in distinguishing the glutinous from the non-glutinous character in the pollen and in the endosperm. The glutinous type of pollen and endosperm is stained reddish-brown while the non-glutinous type is stained blue. The formula for the stock solution of iodine is:

I—5 grams
KI—10 grams
H_2O—500 cc.

This stock solution is diluted to the strengths desired.

The method of crossing

Crosses were made reciprocally in different combinations involving all the varieties listed above. In the actual operation of crossing, two methods were used. The first one was a modification of the method suggested by SHARNGAPANI (1924). The palea and lemma were very gently pulled apart with the fingers and the stamens removed with a pair of fine bent forceps. After pollination, the lemma and palea were brought together and tied with a piece of fine silk thread. The first set of crosses between two Manchurian varieties was made by this method and twelve seeds were obtained as a result of over 2000 pollinations. The method was then

abandoned for two reasons: first, the process is terribly slow, and secondly, the tying of each small spikelet with a silk thread is a painstaking act and very often injures the lemma and palea, resulting very frequently in the shriveling of both.

The second method is quicker and more certain of success. Emasculation was done either very early in the morning before sunrise, or late in the afternoon after sunset, the reason being that the pollen sacs are very sensitive to the touch and may suddenly discharge the pollen grains when emasculation is done under bright sunshine. For some varieties, emasculation had to be performed before the panicle emerged from the leaf-sheath, but for others emasculation had to be made later. Since rice flowers mature from the top panicle downward, all the younger spikelets on the lower part of the panicle were cut off, leaving about 10-15 spikelets at the top. When the anthers reached about half the length of the lemma and palea (this can be seen by holding the spikelet against the sunlight), a very short transverse cut was made near the tip of the spikelet with a pair of very fine scissors. The anthers were carefully removed with a very fine pointed, bent forceps through the transverse cut, the forceps being sterilized with 95 percent alcohol after each movement. Then the panicle was bagged with a glacine paper bag. About fifteen to twenty-four hours later, the emasculated flower was ready to be pollinated, the most receptive stage of the stigma being indicated by the rapid elongation of the filaments remaining after the anthers had been removed. Pollination was done by inserting one or more mature anthers of the desired type into the hollow of the spikelet and dusting the pollen grains into it. The pollinated panicle was then rebagged and tagged with all necessary markings. This method has many advantages over the previous one. First, the process is much quicker. Secondly, pollination may be easily repeated several times on the same flower and is, consequently, made much surer. Thirdly, the time of greatest receptivity of the pistil is indicated by the rapid elongation of the filaments. The only disadvantage of this method is that after the spikelet is cut crosswise at the top, the endosperm of the developing grain grows out of the opening and becomes elongated and slender, instead of retaining its natural plump and strongly compressed shape. However, such grains germinate and grow just as well as the normal type. The following table shows the percentage of success for different periods of pollination, indicating that better results were obtained when pollination was done in the morning than in the afternoon.

Time of pollination	*9–10 A.M.*	*10–11 A.M.*	*11–12 A.M.*	*1–2 P.M.*	*2–3 P.M.*	*3–4 P.M.*	*Total*
Number of spikelets pollinated.....	14	336	532	98	325	182	1484
Number of seeds set.....	9	146	228	25	120	45	573
Percent success..	64.28	43.45	42.85	25.51	37.26	24.72	38.61

The method of recording

The method of keeping pedigree records of the crosses and their successive generations may be illustrated by the example, 101205*DSt*98, where 101 = the first plant of the glutinous variety 100; 205 = the fifth plant of the non-glutinous variety 200; 101205 = F_1 plant, 101 being the pistillate parent and 205 the pollen parent; *D* = the fourth plant; *St* = the starchy class; 98 = the 98th F_2 individual. The total expression, 101205-*DSt*98, then, means the 98th F_2 plant of the starchy class which has come from the fourth F_1 plant of the cross between plant No. 1 of the glutinous variety 100 and plant No. 5 of the non-glutinous variety 200, the former being used as pistillate parent and the latter as pollen parent. This method was found to be very practical in application.

The method of classification

The grains were cut open at their distal ends. After cutting, all the grains from the same plant were put in a Petri dish and enough iodine solution of the proper strength was poured into it to submerge the grains. After a minute or two, the solution was drained off and the red-stained glutinous and the blue-stained non-glutinous grains were picked apart with a pair of forceps. The method was found to be very accurate.

The method of vegetative propagation

When the first growth reached the flowering stage, the depth of water was increased and some solution of ammonium sulphate was added to the pot. This increase of water submerged all the lower nodes of old shoots and very soon new shoots arose from such nodes. The application of nutrients hastens the growth of young stoolings. When the latter reached about the flowering stage, the first growth was ready to be harvested. Then the same process was repeated again and a third growth immediately resulted. Thus, several successive crops could be raised from the same plant. In this way, some of the F_1 plants were kept segregating for two

years without altering their genetic constitution, and interesting results were obtained from successive crops in certain particular lines of study.

EXPERIMENTS AND RESULTS

Distribution of glutinous and non-glutinous grains in F_2 from reciprocal crosses between two Manchurian varieties

The first set of crosses was made in the spring of 1925 between the two Manchurian varieties, designated as 100 and 200 respectively, the former being glutinous and the latter non-glutinous. Twelve F_1 plants were secured the following summer, each giving about the same ratio of glutinous and non-glutinous grains in the F_2 population. With the exception of two cases, all the heterozygous plants showed a deficiency in the glutinous class and three of them gave deficiencies over three times the respective porbable errors. Of the two exceptions, one gave a plus deviation of 9.5 glutinous grains and the other only 2.25; neither, therefore, is significant. The data are presented in table 1.

TABLE 1

12 F_2 progenies from 100×200.

PLANT NUMBER	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	DEVIATIONS	DEVIATION / P.E.	PERCENT GLUTINOUS	RATIO
Progenies each with a deficiency over 3 times the P.E.						
101205D	397	106	−19.75	3.01	21.07	3.16:0.84
103205A	638	168	−33.50	4.04	20.84	3.17:0.83
104207A	297	68	−23.25	4.16	18.63	3.25:0.75
Total	1332	342	−76.50	6.40		
Progenies with no significant deviation						
101205A	1176	373	−14.25	1.24	24.08	3.04:0.96
101205B	743	234	−10.25	1.12	23.95	3.04:0.96
101205C	1142	378	− 2.0	0.17	24.86	3.01:0.99
101205E	592	171	−19.75	2.44	22.41	3.10:0.90
101205F	426	133	− 6.7	0.907	23.79	3.05:0.95
101205G	599	190	− 7.25	0.88	24.06	3.04:0.96
205101A	382	140	9.5	1.42	26.81	2.93:1.07
103205B	611	172	−23.75	2.9	21.96	3.12:0.88
103205C	693	234	2.25	0.253	25.24	2.99:1.01
Total	6364	2025	−95.70	3.57	24.13	
Grand total	7696	2367	−148.75	5.08	23.52	3.06:0.94

A brief analysis of the data in table 1 reveals three important points. First, each of the twelve segregating plants gives approximately a 3:1 ratio, thus strengthening a previous statement that the glutinous character is due to a simple Mendelian recessive gene. Secondly, there is a total deficiency in the glutinous class about 5.08 times the probable error which appears to be significant, for the odds against the occurrence of such a deviation being due to chance alone are over 1341 to 1. Thirdly, this large deficiency in the totals appears to be due to the accumulation of the ten smaller ones in the same direction. Attention may also be called to the plant 104207A, which gave only 18.63 percent glutinous grains instead of 25.0 percent.

Further data from the successive crops of this plant, however, showed that it produced 26.62 percent and 26.23 percent glutinous grains in the second crop and fourth crop respectively (see table 6). This means that though only 18.63 percent glutinous grains were produced in the first crop, it retained the potentiality for producing the percentage expected on Mendelian grounds. In the light of these facts, it seems to be justifiable to state that the gene in question is somewhat plastic in its function under different conditions and that the glutinous material in the 12 sister progenies recorded in table 1 is in all probability homogeneous.

Comparison of F_2 distribution in successive crops of 100×200

The data presented in table 1 agree very closely with those of PARNELL (1921). This agreement, however, does not imply that the results of other earlier investigators are inaccurate. Indeed, it was through the attempt to find out how the fluctuations of those workers might have arisen that the writer resorted to a new channel of approach. A method was developed, as described previously, of propagating the heterozygous plants by vegetative means. It was thought that with the genetic constitution remaining constant, any difference in the ratio of the glutinous and non-glutinous grains in successive crops raised by vegetative propagation might be attributed to the effect of the environment on the behavior of the gene in question; thus, by comparing the segregation in successive crops, a glimpse might be gained as to how the glutinous gene responds under different conditions. Mention may be made here that during the second growth, five of the twelve F_1 plants, namely, 101205A, 101205C, 101205G, 103205A and 205101A were lost. Eight more crops were raised from the remaining seven plants. The data are presented in table 2.

TABLE 2

Comparison of segregation in successive crops of 100×200.

CROP	TIME OF HARVESTING	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEV. / P.E.	PERCENT GLUTINOUS
First	9/21/25	7696	2367	10063	−148.75	5.08	23.52
Second	10/30/25	852	293	1145	6.75	0.46	25.58
Third	1/18/26	227	87	314	8.5	1.64	27.70
Fourth	4/ 9/26	1466	491	1957	2.75	0.21	25.08
Fifth	5/14/26	439	148	587	1.25	0.17	25.21
Sixth	6/28/26	1373	488	1861	22.75	1.80	26.22
Seventh	7/29/26	1478	464	1942	−21.5	1.74	23.89
Eighth	8/31/26	1141	371	1512	− 7.0	0.61	24.53
Ninth	10/23/26	1457	452	1909	−25.25	1.97	23.67
Total		16129	5161	21290	−161.5	3.79	24.24

Generally, the grain matures about thirty days after pollination. A chronological analysis of table 2 reveals the fact that all pollination in the period beginning from October 1, 1925, to the last of May 1926, resulted in the production of over 25 percent glutinous grains in every case, while all pollinations in the period from the latter part of June to the latter part of September resulted in the reverse. To put it in more explicit terms, the glutinous gene with the reaction of its associates gave rise to the production of 23.52 percent glutinous grains under the conditions prevailing when the first crop was raised; the same genetic complex, however, produced more than 25 percent consecutively in the five succeeding crops raised in the second period and, in the following period, again, less than 25 percent of glutinous grains was produced consecutively in three crops. These orderly periodical changes may be taken to indicate that while the glutinous gene itself was not changed under different conditions, its physiological effect was altered more or less as shown by the end results. While it is entirely conceivable how the earlier investigators got fluctuations in their results in the light of the present data, conclusion in this respect will be deferred until further data are presented.

Distribution of F_2 classes from 14 combinations of unrelated forms

In the summer of 1925, numerous crosses were made involving ten unrelated glutinous and non-glutinous varieties obtained from different countries. As a result, nine F_1 plants were secured from the combination

421×104; fifteen from 600×100; seven from 300×600; four from 1315×619; five from 903×619; five from 300×400; three from 421×1316; and two from each of the following combinations, namely, 903×715, 906×706, 320×705, 306×800b$_4$ and 1000b$_7$915. F_2 generations were raised in 1926 from all these combinations. In every case, the non-glutinous was dominant over the glutinous in the hybrid and the two types of grains were segregated out in an approximately 3:1 ratio in the F_2. The results are summarized and presented in table 3.

TABLE 3

Segregation from F_2 populations of 14 combinations of unrelated varieties.

COMBINATIONS	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEV. / P. E.	PERCENT GLUTINOUS	RATIO
100×200	16129	5161	21290	−161.5	3.79	24.24	
421×104	7185	2313	9498	−61.5	2.16	24.35	
4269×4957	4748	1471	6219	−83.75	3.63	23.65	
300×600	1263	373	1636	−36.0	3.05	22.80	
1315×619	445	137	582	− 8.5	1.21	23.54	
903×619	1435	428	1863	−37.75	2.99	22.97	
300×400	1143	338	1481	−32.25	2.87	22.82	
903×713	443	154	597	+ 4.75	0.66	25.80	
906×706	574	163	737	−21.25	2.68	22.12	
320×705	446	132	578	−12.50	1.78	22.83	
421×1316	179	60	239	+ 0.25	0.05	25.06	
1000b$_7$×915	229	75	304	− 1.0	0.19	24.67	
306×800b$_4$	244	72	316	− 7.0	1.34	22.78	
600×100	9580	3005.00	12585	−141.25	4.31	23.87	
Total	44043	13882	57925	−599.25	8.52	23.96	3.04:0.96
Expected	*43443.75*	*14481.25*	*57925*	*0*	*0*	*25.00*	*3:1*

A glance at the data in table 1 and those in table 3 reveals their strong resemblance in regard to the deficiency in the glutinous class, in spite of the fact that the former represents the segregates from twelve plants having the same parents, while the latter involves fourteen combinations of unrelated varieties. Of these fourteen combinations, only two showed a slight plus deviation, namely, 4.75 and 0.25 grains respectively, both being, however, small enough to be negligible. All the rest gave a greater or lesser deficiency. The net deficiency amounts to 599.25 grains. Such a deviation is about 8.52 times the probable error. The odds against the occurrence of a deviation as great as this on the basis of chance alone is over fourteen

million to one. These data, therefore, further strengthen the three cardinal points indicated by the data in table 1.

Corroborative evidence for the accumulation of deficiencies

Attention has already been called to the fact that the large deficieny in the totals is due to the accumulation of many smaller ones in the same direction. This statement is well supported by the data presented in table 1, where each of the nine progenies shows no significant deviation, yet there is a deficiency of 95.7 glutinous grains in the totals which is 3.57 times the probable error. A similar situation is also exhibited in the data presented in table 3. Further corroborative evidence for the accumulation of small deficiencies was obtained in the cross 4269×4957. Five crops were raised by vegetative propagation. None of them gave a large deviation, yet a total deficiency equal to 3.63 times the probable error arises as shown in table 4.

TABLE 4

Accumulation of the deficiency of glutinous grains in the F_2 generation of 4269×4957.

CROP	TIME OF HARVESTING	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEV. / P. E.	PERCENT GLUTINOUS
First	4/26/26	661	190	851	−22.75	2.67	22.32
Second	5/14/26	249	71	320	− 9.0	1.72	22.18
Third	6/24/26	844	243	1087	−28.75	2.88	22.35
Fourth	7/23/26	912	310	1222	4.5	0.44	25.36
Fifth	10/ 8/26	2082	657	2739	−27.75	1.81	23.98
Total		4748	1471	6219	−83.75	3.63	23.65

These data are further supplemented by the results obtained from the sixth crop of the combination 600×100. Of the 14 segregating progenies none had a deviation over 2.5 times the probable error. Yet, the total deficiency of glutinous grains amounted to 4.1 times the probable error as shown in table 5.

Cause of the deficiency of glutinous grains

As in all biological researches, when a phenomenon is observed and its reality is subsequently ascertained in explicit terms, the causal agent is next to be sought for; so it is with the present case. The glutinous and non-glutinous characters behave as a contrasting pair of hereditary traits. Breeding facts presented in the preceding pages have shown that they

TABLE 5

Segregating F_2 progenies showing the accumulation of deficiency.

PLANT NUMBER	STARCHY GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEVIATION / P. E.
Progeny with a deficiency over 3 times the probable error					
61010B	73	13	86	− 8.5	3.13
Progencies with no significant deviation					
610101A	82	21	103	− 4.75	1.60
610101C	151	38	189	− 9.25	2.30
611103A	145	36	181	− 9.25	2.35
611103B	123	34	157	− 5.25	1.43
611103C	24	12	36	3	1.71
611103D	133	34	167	− 7.75	2.05
602103A	104	35	139	0.25	0.07
602103B	124	34	158	− 5.5	1.49
608103A	86	23	109	− 4.25	1.39
608104A	69	24	93	0.75	0.26
608104B	83	25	108	− 2.0	0.65
608104C	90	30	120	0	0
615103A	47	11	58	− 3.5	1.57
615103B	178	54	232	− 4.0	0.89
Total	1439	411	1850	−51.5	4.10
Grand total	1512	424	1936	−60	4.68

are due to a pair of allelomorphic genes, G_l g_l, thus giving a simple Mendelian ratio. The data further indicate, beyond reasonable doubt, that there is a significant deficiency in the recessive or glutinous class. The fact that this large deficiency is due to the accumulation of many smaller ones in the same direction has also been ascertained. The question now, is to determine the casual agent that is responsible for the small deviations which, in turn, lead to the significant deficiency. Attempts have been made through the following channels.

Accuracy of classification

It was thought that the small deviations which lead to a significant deficiency of glutinous grains might be due to misclassification. To search for evidence of this kind, all grains from progeny 101205D, which gave a large deficiency as presented in table 1, were planted for further analysis. Due to the damage by cutting in the first classification, only 434 plants were raised out of the original 503 F_2 grains. When these plants reached the

flowering stage, each was examined in duplicate by staining the pollen with iodine solution. As a result, it was found that all the 87 plants arising from grains which had been put in the glutinous class produced homogenous glutinous pollen, while those plants arising from grains that had been classified as non-glutinous comprised two types. One of these types, of which there were 118 plants, produced homogeneous non-glutinous pollen while the other type comprising 229 plants, segregated for glutinous and non-glutinous pollen, the former stained reddish-brown the latter deep blue. This shows that the classification of the F_2 grains was very accurate. Consequently, the attempt to account for the deficiency of glutinous grains on that basis is fruitless.

Effect of seasonal infertility

Under proper environment, when pollinations are successful, all, or practically all spikelets on the pancile are filled, though a few of them may be empty. When the environment is adverse, however, a great percentage of the spikelets remains completely empty, though all pollen grains are still normal, showing that the empty spikelets, in this case, are due to environmental factors rather than to genetic ones. It was early noted that the temperature and humidity fluctuated very much in the greenhouse where the F_1 plants were grown. Parallel to this fluctuation to temperature and humidity was the fluctuation of the percentage of empty spikelets in successive crops of the same plant raised by vegetative propagation. To those empty spikelets due to unfavorable environmental factors not definitely known the term "seasonal infertility" is here applied. It was thought that the high percentage of seasonal infertility of this kind might effect the percentage of glutinous grains in the opposite direction. Attempts were then made to propagate many F_1 plants by vegetative means and collect the fertile and infertile spikelets in successive crops from the same plant or from the same combination of crosses. These data were tabulated to compare with the percentage of glutinous grains for which the plant or combination of crosses in question was segregating. The results are presented in table for the plant 104207A, table 7 for 421×104, table 8 for 600×100, table 9 for 100×200, for table 10 for 4269×4957, respectively.

Table 6 shows that when the seasonal infertility is 31.85 percent, the glutinous class amounts to 26.62 percent in the second crop. In the fourth crop, when infertility gets as high as 56.88 percent, the percentage of glutinous grains still remains about 26. No emphasis can be laid on the third crop because of the small numbers. While these crops seem to

indicate that the glutinous percentage tends to increase with that of seasonal infertility the sixth crop shows the inconsistency, so no definite correlation is indicated by these data.

TABLE 6

Effect of seasonal infertility on the starchy and glutinous ratio in progeny (104×207)A.

CROP	TIME OF HARVESTING	NUMBER OF FERTILE SPIKELETS			NUMBER OF INFERTILE SPIKELETS	PERCENT INFERTILITY	PERCENT GLUTINOUS	DEV. / P.E.
		Non-glutinous	Glutinous	Total fertile				
First	9/21/25	297	68	365			18.63	4.16
Second	10/30/25	124	45	169	79	31.85	26.62	.72
Third	1/18/26	5	4	9	63	67.5	44.44	.63
Fourth	4/ 9/26	194	69	263	347	56.88	26.23	.61
Fifth	5/14/26	50	15	65	23	26.13	23.07	.16
Sixth	6/28/26	194	53	247	165	40.04	21.45	1.79
Seventh	7/29/26	131	43	174	49	21.97	24.71	0.13
Eighth	8/31/26	93	30	123	38	23.60	24.39	0.23
Ninth	10/23/26	1	1	2	8	80.00	50.00	0.38

Table 7 shows that when the percentage of seasonal infertility is below 50, the glutinous percentage is below 25 percent, and that when infertility gets as high as 77.55 percent, the glutinous ratio increases to more

TABLE 7

Effect of seasonal infertility on the starchy and glutinous ratio in progeny (421×104).

CROP	TIME OF HARVESTING	NUMBER OF FERTILE SPIKELETS			NUMBER OF INFERTILE SPIKELETS	PERCENT INFERTILITY	PERCENT GLUTINOUS	DEV. / P.E.
		Non-glutinous	Glutinous	Total fertile				
First	11/13/25	420	134	554	399	41.86	24.18	0.65
Second	1/18/26	27	9	36	128	78.04	25.0	0
Third	4/14/26	359	142	501	1731	77.55	28.34	2.56
Fourth	5/14/26	243	80	323	2499	88.55	24.76	0.13
Fifth	6/28/26	1401	429	1830	1954	51.63	23.44	2.28
Sixth	8/ 7/26	1285	397	1682	852	33.62	23.60	1.97
Seventh	9/ 4/26	883	278	1161	465	28.59	23.94	1.27
Eighth	10/23/26	2567	844	3411	3353	49.57	24.74	0.51
Total		7185	2313	9498	11381	54.50	24.35	2.16

than 28 percent. While there is an inconsistent tendency for the parallel increase of the percentage of seasonal infertility and that of glutinous

grains, there is no evidence whatever that high seasonal infertility may cause the deficiency of glutinous grains.

TABLE 8

Effect of seasonal infertility on the starchy and glutinous ratio combination of 600×100.

CROP	TIME OF HARVESTING	NUMBER OF FERTILE SPIKELETS			NUMBER OF INFERTILE SPIKELETS	PERCENT INFERTILITY	PERCENT GLUTINOUS	DEV. / P.E.
		Non-glutinous	Glutinous	Total fertile				
First	10/30/25	723	270	993	432	30.31	27.19	2.36
Second	1/18/26	210	78	288	887	75.48	27.08	1.21
Third	4/ 9/26	618	188	806	1010	55.61	23.32	1.62
Fourth	5/14/26	129	41	170	29	14.57	24.12	0.39
Fifth	6/28/26	1400	413	1813	508	21.88	22.77	3.24
Sixth	8/31/26	1512	424	1936	466	19.40	21.90	4.68
Seventh	10/23/26	4988	1591	6579	4238	39.17	24.18	2.26
Total		9580	3005	12585	7570	37.55	23.88	4.31

Table 8 shows that when seasonal infertility is as low as 19 percent, as in the sixth crop, there is a large deficiency of glutinous grains which is very likely a significant deviation. On the other hand, when infertility

TABLE 9

Effect of seasonal infertility on the starchy and glutinous ratio combination of 100×200.

CROP	TIME OF HARVESTING	NUMBER OF FERTILE SPIKELETS			NUMBER OF INFERTILE SPIKELETS	PERCENT INFERTILITY	PERCENT GLUTINOUS	DEV. / P.E.
		Non-glutinous	Glutinous	Total Fertile				
First	9/21/25	7696	2367	10063			23.42	5.08
Second	10/30/25	852	293	1145	596	34.23	25.58	0.46
Third	1/18/26	227	87	314	1046	76.91	27.70	1.64
Fourth	4/ 9/26	1466	491	1957	1971	50.17	25.08	0.21
Fifth	5/14/26	439	148	587	131	18.24	25.21	0.17
Sixth	6/28/26	1373	488	1861	732	28.23	26.22	1.80
Seventh	7/29/26	1478	464	1942	477	19.71	23.89	1.74
Eighth	8/31/26	1141	371	1512	441	22.58	24.53	0.61
Ninth	10/23/26	1457	452	1909	1792	48.41	23.67	1.97
Total		16129	5161	21290	7186	25.23	24.24	3.79

reaches 75 percent, as in the second crop, the glutinous class contains as high as 27 percent of the population where 25 percent only is expected in

normal segregation. This again lends no support to the idea that high seasonal infertility causes the deficiency of the recessive type.

Table 9 shows that when the percentage of seasonal infertility is highest (76.91) percent, as is the case in the third crop, the percentage of glutinous grains is also highest (27.7) percent.

TABLE 10

Effect of seasonal infertility on the starchy and glutinous ratio in progeny (4269×4957).

CROP	TIME OF HARVESTING	NUMBER OF FERTILE SPIKELETS			NUMBER OF INFERTILE SPIKELETS	PERCENT INFERTILITY	PERCENT GLUTINOUS	DEV. / P. E.
		Non-glutinous	Glutinous	Total fertile				
First	4/26/26	661	190	851			22.32	2.67
Second	5/14/26	249	71	320	214	40.07	22.18	1.72
Third	6/24/26	844	243	1087	2785	71.92	22.35	2.88
Fourth	7/23/26	912	310	1222	3524	74.25	25.36	0.44
Fifth	10/ 8/26	2082	657	2739	2621	48.89	23.98	1.81
Total		4748	1471	6219	9144	59.52	23.65	3.63

Table 10 shows that when the percentage of seasonal infertility is lowest (40.07 percent) the percentage of glutinous grains is also lowest (22.18 percent) as is the case in the second crop. On the other hand, when the percentage of infertility is highest (74.25 percent) the percentage of glutinous grains is also the highest (25.36 percent) in the series. This can not be taken as a mere coincidence because the tendency is manifested in the preceding four tables also. After careful analysis of these data, as presented in the preceding five tables (6-10), no satisfactory evidence is found to support the view that the high percentage of seasonal infertility affects the percentage of glutinous grains in the opposite direction. In the light of these data, it seems to be justifiable to state that it is extremely improbable that high seasonal infertility will give rise to the significant deficiency either by diminishing the union of glutinous gametes, or by eliminating a certain proportion of glutinous zygotes.

On the other hand, the same body of data indicates a strong tendency for the parallel increase of the percentage of seasonal infertility and that of glutinous grains in segregating plants. This parallel increase cannot be taken as a mere coincidence. Although the meaning of this parallel increase is unknown, it is conceivable that whatever the conditions are, that cause the high percentage of seasonal infertility they may be, at the same time, advantageous for the activity of the glutinous gene.

Normality of segregation at microsporogenesis

It was thought that the small deviations that lead to a significant deficiency of glutinous grains might be due to some irregularity of chromosome behavior at sporogenesis causing the production of fewer spores that carry the glutinous gene. Unpublished cytological studies by the author show that the meiotic divisions in the pollen mother cells are normal at least in three of the unrelated varieties, namely, 1000, 200, and 1300, the first being diploid and the last two possibly tetraploid. More convincing evidence for the normality of microsporogenesis, however, came from the study of the glutinous and non-glutinous pollen grains which represent the

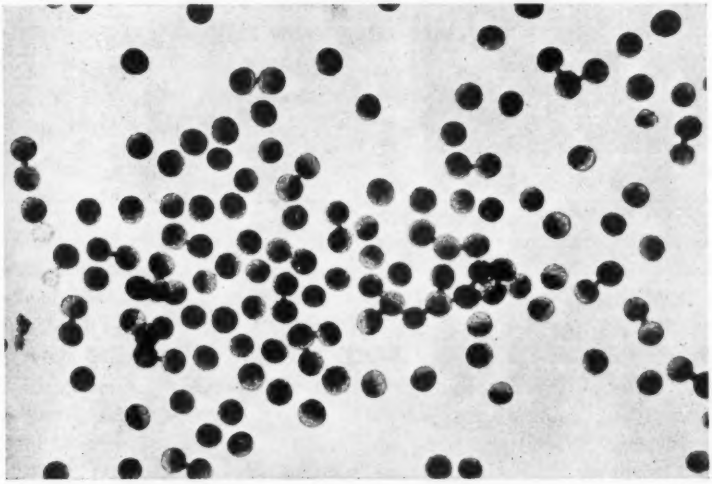


FIGURE 1.—Segregation of non-glutinous and glutinous pollen grains, the former being darker than the latter.

direct results of Mendelian segregation. This was done by counting the two types of pollen, one staining blue and the other reddish-brown in the same anther (figure 1) from segregating plants. The results are presented in table 11.

Of the 6330 pollen grains counted, 3179 were non-glutinous and 3151 were glutinous, while 3165 pollen grains of each type are expected on the basis of a 1:1 ratio. There is a deficiency of fourteen glutinous pollen grains. This deviation is, however, only 0.51 times the probable error. It seems justifiable, therefore, to consider that the two types of pollen are formed with equal frequency.

TABLE 11

Frequency of glutinous and non-glutinous pollen grains.

F_1 PLANTS USED	NUMBER OF ANTHERS EXAMINED	NUMBER OF MICROSCOPIC FIELDS COUNTED	NUMBER OF NON-GLUTINOUS POLLEN GRAINS	NUMBER OF GLUTINOUS POLLEN GRAINS	TOTAL OF EACH TYPE IN THE SAME PLANT	
					Non-glutinous	Glutinous
104207A	1	21	306	318		
	1	12	137	117	443	435
608104A	1	21	180	213		
	1	24	434	405	614	618
608104C	1	25	185	169		
	1	38	230	239	415	408
610101C	1	38	347	325		
	1	36	309	313	656	638
611103A	1	23	222	210		
	1	36	291	287	513	497
608103A	1	25	288	316		
	1	26	250	239	538	555
6 plants	12	325	3179	3151	6330	
Expected			*3165*	*3165*	*6330*	

Possibility of an accessory factor

To account for the disturbed waxy ratio in maize, MANGELSDORF and JONES (1926) advanced the hypothesis that a gametophyte factor, G_a, is present on the waxy chromosome and that this accessory factor disturbs the waxy and non-waxy ratios by causing differential pollen tube growth. Whether this hypothesis is the true explanation for the disturbed ratio in maize, remains to be proven. It is with this hypothesis in mind, however, that the writer further analyzes the situation of the disturbed glutinous and non-glutinous ratio in rice.

It may be pointed out that on the assumption mentioned above, we may have three kinds of combinations in the original crosses in regard to the G_l g_l pair, namely:

1. $G_a\ g_l \cdot G_a\ g_l \times g_a\ G_l \cdot g_a\ G_l$
2. $G_a\ g_l \cdot g_a\ g_l \times G_a\ G_l \cdot g_a\ G_l$
3. $g_a\ g_l \cdot g_a\ g_l \times G_a\ G_l \cdot G_a\ G_l$

If the G_a factor stimulates pollen tube growth, we should expect that from the first combination every F_1 plant ($G_a\ g_l g_a\ G_l$) will produce more than 25 percent glutinous grains; from the second combination, some F_1 plants will produce an excess of recessives and others a deficiency of recessives; and from the third combination, every F_1 plant ($G_a G_l g_a g_l$) will produce less than 25 percent of glutinous grains. Since practically all crosses involving many unrelated varieties of rice gave a deficiency of glutinous grains as reported in the preceding pages, the first combination is entirely eliminated and we shall consider the possibility of the last two combinations only. In this consideration, an arbitrary limit is made such that a plus deviation over three times the probable error will be classified as "high recessive" and a minus deviation of the same size as "low recessive," while a deviation less than three times the probable error in either direction will be considered as "normal recessive." It should be pointed out that such a standard is already very low, considering the nature of the problem in hand, although MANGELSDORF and JONES, in their classification, use a smaller deviation (twice the probable error), as the criterion. The justification for such practice will be discussed below.

Evidence from the F_2 *generation.* It was found that among 58 F_1 plants, each producing from two to nine successive crops through vegetative propagation under different environmental conditions, only three plants ever gave an excess of glutinous grains showing a plus deviation over three times the probable error. These were 103205B, 101205B, and 611103D. The data for the plant 103205B are presented in table 12. In its sixth crop,

TABLE 12

Successive crops of 103205B.

CROPS	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	DEVIATION	DEVIATION / P. E.	PERCENT GLUTINOUS
First	611	172	−23.75	2.9	21.96
Second	123	39	− 1.5	0.40	24.07
Third	5	4	1.75	2.01	44.44
Fourth	223	76	1.25	0.24	25.41
Fifth	121	40	− 0.25	0.67	24.84
Sixth	239	106	19.75	3.64	30.72
Seventh	257	74	− 8.75	1.64	22.35
Eighth	235	85	5.0	0.95	26.56
Ninth	512	162	− 6.5	0.85	24.03
Total	2326	758	−13.0	0.80	24.59

30.72 percent glutinous grains were produced. It is to be noted, however, that the same plant produced 21.96 percent of recessives in the first crop showing a deficiency of 2.9 times the probable error. If this plant were really "high recessive," how could such a large deficiency occur in the first crop? Indeed, if only the data from the first crop were availabe, this plant might be classified as "low recessive" according to our standard.

The data for the plant 101205B are presented in table 13. Here, 101205B produced in its sixth crop an excess of glutinous grains giving a plus deviation over six times the probable error. But the number is so small that the deviation can not be considered seriously. This is further indicated by the tendency toward deficiency in the first and fourth crops where the numbers are considerably larger.

TABLE 13

Successive crops of 101205B showing excess of recessives in sixth crop.

CROP	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	DEVIATION	DEVIATION / P. E.	PERCENT GLUTINOUS
First	743	234	−10.25	1.12	23.95
Second	127	39	− 2.5	0.66	23.49
Third	55	18	− 0.25	0.03	24.65
Fourth	208	67	− 1.75	0.36	24.36
Fifth	34	15	2.75	1.34	30.61
Sixth	58	43	17.75	6.03	42.57
Seventh	15	7	1.5	1.09	31.81
Eighth	134	50	4.0	1.01	27.17
Ninth	16	7	1.25	0.89	30.43
Total	1390	480	12.5	0.98	25.66

There remains to be considered the data for the plant 611103D. From the combination of 600×100 fifteen sib F_1 plants were obtained, each of which produced seven successive crops. Only 611103D, however, produced a plus deviation, that is over three times the probable error, as shown in table 14. Furthermore, this excess of recessives was given only in the seventh crop of the plant, as shown in table 15, whereas in its sixth crop, a deficiency of 2.05 times the probable error actually occurred. Indeed, according to the standard of MANGELSDORF and JONES, this plant would have been classified as "low recessive," if we had not obtained further data like that of the seventh crop. On the other hand, the plant, 610101B, producing a slight excess of recessives in the first crop, later gave a significant deficiency of reccessives in the seventh crop, as shown in table 14.

TABLE 14

Sister F_2 progenies from the combination 600×100(7th crop).

PLANT NUMBER	STARCHY GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEVIATION / P. E.
Progeny with an excess over 3 times the probable error					
611103D	306	134	440	24	3.92
Progeny with a deficiency over 3 times the probable error					
610101B	333	77	410	−25.5	4.31
Progenies with no significant deviation					
611103A	184	67	251	4.25	0.91
611103B	56	15	71	− 2.75	1.11
611103C	110	35	145	− 1.25	0.35
610101A	468	139	607	−12.75	1.77
610101C	381	126	507	− 0.75	0.11
602103A	196	72	268	5.00	1.04
602103B	434	134	568	− 8.00	1.14
608103A	275	77	352	−11.00	2.00
608104A	136	35	171	− 7.75	2.03
608104B	236	70	306	− 6.5	1.27
608104C	252	75	327	− 6.75	1.27
615103A	631	200	831	− 7.75	0.92
615103B	990	335	1325	3.75	0.35
Total	4349	1380	5729	−52.25	2.35
Grand total	4988	1591	6579	−53.75	2.26

TABLE 15

Successive crops of 611103D showing excess of recessives in the seventh crop.

CROP	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEVIATION / P. E.
First	47	14	61	− 1.25	0.55
Second	10	3	13	− 0.25	0.24
Third	46	16	62	0.5	0.21
Fourth	..	..	..	..	..
Fifth	64	22	86	0.5	0.11
Sixth	133	34	167	− 7.75	2.05
Seventh	306	134	440	24.00	3.92
Total	606	223	829	15.75	1.87

In the light of these facts, it is justifiable to state that among the 58 F_1 plants studied, there is no positive evidence of one that will produce "high recessives" in the F_2 generation as would be expected from the second combination, $G_a\ g_l \cdot g_a\ g_l \times G_a\ G_l \cdot g_a\ G_l$. On the other hand, the few cases cited like plants 103205B and 611103D, that might have supported the hypothesis with their figures from the sixth crop and seventh crop, respectively, actually offset the excess by the production of deficiencies in other crops.

Evidence from F_3 *generation.* Ten F_1 plants were obtained from the cross 4269×4957 made at the RICE EXPERIMENT STATION, Crowley, Louisiana, in the summer of 1925. From these ten F_1 plants we get a total F_2 population of 6219 grains of which 4748 were non-glutinous and 1471 were glutinous, giving a minus deviation that is 3.63 times the probable error. Ninety-seven large segregating F_3 progenies were obtained and the data are presented in table 16. Of these 97 progenies, two gave an excess of glutinous grains with a deviation over three times the probable error and three produced a deficiency of recessives each with a minus deviation over three times the probable error, while the remaining ninety-two progenies gave no significant deviation. It is to be noted that the progeny CSt_{32}, which gave a minus deviation over three times the probable error had only 126 grains and, therefore, it should not be considered very seriously. Leaving this progeny out for the time being, we have only four progenies to consider, namely, ESt_{47}, FSt_{38}, ASt_{33}, and ISt_{40}. The former two progenies produced "high recessives," while the latter two gave "low recessives."

While these progenies may be cited in favor of the combination, $g_a\ g_l \cdot g_a\ g_l \times G_a\ G_l \cdot G_a\ G_l$, the evidence is very weak, for the ratio of high, normal, and low recessives is 2:92:2. On the basis of chance alone, the probable occurrence of a deviation as great as three times the probable error in 100 trials is 4.30, and we have four cases out of 97. It seems to be justifiable to consider this occurrence to be within the limits of random sampling.

There is no experimental evidence in support of the hypothesis that the deficiency of glutinous grains is caused by an accessory factor linked with the glutinous gene.

In this connection, attention may again be invited to the fact that the small minus deviations of the ninety-two F_3 progenies in table 16 finally lead to one as large as 142 individuals, which is practically three (2.96) times the probable error. It is this sort of constancy in the occurrence o small diviations that seems to be the significant thing in the last analysis.

DISCUSSION

Different from the ordinary blue-staining starch is the "red-staining starch" found in the waxy maize. The latter exists also in other genera of Gramineae. GRIS (1860) found it in *Oryza sativa* var. *glutinosum*. MEYER (1886) found it in *Panicum miliaceum* var. *canditum glutinosum* and *Sorghum vulgare* var. *glutinosum*. KEMPTON (1921) observed it in *Coix lachryma jobi*. It is of theoretical interest that five genera of Gramineae have this type of starch. It must be borne in mind, however, that the indentity of the materials has not been proved in all these cases.

The typical differences between glutinous and common rice starches is found in the iodine reaction, in which the glutinous rice starch shows a red brownish color, while the common rice starch gives a blue color. TANAKA (1907) thinks that the red coloration of the former is due to a characteristic property of the starch itself and denies DAFERT and MEYERS'S contamination theory of amylopectin, erythrodextrin, or special albuminoids. TADOKORO and SATO (1923) find many characteristic differences between the colloidal properties of glutinous and non-glutinous rice starches, but no noticeable differences between their ordinary chemical properties. They conclude, therefore, that the glutinous rice starch and the non-glutinous rice starch are not necessarily different substances, but seem to be only different in degree of polymerization.

Whatever the two types of color reaction may mean, they serve as excellent criteria in the classification of breeding material. When the red-staining glutinous type is crossed with the blue-staining non-glutinous type, the immediate F_1 is fully blue-staining exactly like the non-glutinous parent. When the F_1 plants reach the stage of sporogenesis, two types of pollen (one red-staining and the other blue-staining) are formed with equal frequency. On self-pollinating such individuals, we obtained an F_2 population of 57,925 individuals comprising 44,043 blue-staining and 13,882 red-staining grains, thus giving a ratio of 3.04: 0.96. Since this behavior corresponds so closely with the Mendelian phenomenon of segregation and independent assortment of genes, it is very safe to attribute the non-glutinous-glutinous character to a pair of Mendelian allelomorphs, G_l g_l, On this basis, we should expect 25 percent glutinous grains in F_2, but actually we get only 23.965 leaving a deficiency of 1.035 percent. While in a small population, a deficiency of one percent may not be noticeable, in a population as large as ours, a 1.035 percent deviation means 599.25 individuals, or 8.52 times the probable error. This deficiency is in all probability of biometrical significance, for the odds against the

occurrence of such a deviation on the basis of chance alone is over fourteen million to one. Sufficient data have been brought forward to show that this significant deficiency is due to the accumulation of many small deviations in the same direction. Almost every set of data presented in the preceding pages indicates such a tendency, but table 5 embodies one of the most clear-cut examples. Indeed, it is these small deficiencies that, in the writer's opinion, demand our serious attention in the attempt to account for the significant deficiency in the totals, the reasons being three fold. First, their pre-existence in an almost constant manner foreshadows the end result. Secondly, their tendency to accummulate in the same direction under normal environmental conditions indicates an intrinsic causal agent. Lastly their small size, so small as to appear insignificant in individual cases, indicates that the agent concerned has a very slight differential effect.

In searching for the differential agency which causes the small deviations several possibilities have been considered. Experimental results in the F_3 generation show that the classification of F_2 grains is very accurate. There is little probability, therefore, that the deviations are due to systematic errors in classification.

Numerous counts of pollen grains show that the glutinous and non-glutinous microspores are formed in equal numbers in F_1 plants having the constitution of $G_l\ g_l$. This demonstrates that the process of sporogenesis is normal.

The study of the relation between the percentage of infertile spikelets and that of the glutinous grains in plants segregating for this character sheds no light on the cause of the deficiency. The spikelet is either entirely empty or completely filled. It is to be noted that the infertility in question is due to environmental factors and fluctuates in amount under different conditions. If it were the causal agent of the deficiency of glutinous grains, we should expect that the higher the percentage of seasonal infertility the greater the deficiency. But this is not the case. The percentage of glutinous grains tends to show a parallel increase with the percentage of infertility.

While all these researches have not directly disclosed the causal agent they, by no means, lead us to despair, for they have actually helped to narrow the problem down to a specific stage. The life cycle of a plant in its essential features consists of four phases, namely, sporogenesis, gametophyte development, fertilization, and the ontogeny of the sporophyte. That the two types of microspores in segregating plants are equal in number and filled with carbohydrate reserves shows that the de-

ficiency is not occasioned before or during sporogenesis. The spikelets are either entirely empty, or completely filled with no grains of intermediate character which indicates that the deficiency is not occasioned after fertilization through selective elimination of certain glutinous zygotes during the ontogeny of the sporophyte. The fact that under one set of environmental conditions a given plant may produce a deficiency of glutinous grains and under other circumstances an excess, makes it improbable that selective fertilization is the cause of the deficiency of glutinous grains. By eliminating three of the four phases in the life cycle of a plant, we are led to seek for the cause of the deficiency of glutinous grains in the development of the gametophyte.

As already stated, COLLINS and KEMPTON (1911) and KEMPTON (1919) have obtained a significant deficiency of waxy grains in maize when plants heterozygous for this character are selfed. A similar deficiency occurs when pollen of a heterozygote is applied to the recessive waxy ear, whereas the reciprocal combination results in a small excess of waxy kernels. To account for this difference, BRINK and MACGILLIVRAY (1924) suggest a differential rate of pollen tube growth. BRINK (1925) has furnished some evidence for the slower growth of the waxy pollen tube in his experiments on short and long silks, though this is not corroborated in a later study (BRINK, 1927). In rice where it is not practical to make backcrosses, the significant deficiency of glutinous grains has only been obtained on selfing plants heterozygous for the glutinous character. Utilizing the evidence from the rice, as well as that from maize, we may note the following relations:

A significant deficiency of glutinous grains has been proven beyond reasonable doubt by an enormous body of breeding results. This deficiency has been demonstrated to be due to the accumulation of many small deviations in the same direction. These small deficiencies—so small as to appear negligible in individual cases—have foreshadowed that their causal agent has a very slight differential effect. By tracing through the life cycle of the plant, it is shown that the differential cause in question is in all probability to be sought during the stage of the gametophyte development. In maize, reciprocal backcrosses have shown that it is the male gametophyte that plays the differential role. In the male gametophyte the most vigorous vegetative activity occurs during pollen tube growth. In heterozygous rice plants having the constitution $G_l\, g_l$, there are two types of pollen tubes in competition. In the light of these facts, it is reasonable to postulate that the significant deficiency of glutinous grains is occasioned by the slower growth of the glutinous pollen tube.

Now, to what is the slower growth of the glutinous pollen tube due? In maize MANGELSDORF and JONES (1926) postulated an accessory factor, G_a, on the waxy chromosome pair stimulating the rate of pollen tube growth. Thus, the combination $G_a w_x$ will give excess of recessives, while $g_a\ w_x$ will produce a deficiency of recessives. Ingenious as this hypothesis may appear to be, it is inapplicable to the present case. It has been pointed out that on this assumption, we may have only three kinds of combinations in the original crosses in regard to the $G_l\ g_l$ pair, namely:

1. $G_a\ g_l \cdot G_a\ g_l \times g_a\ G_l \cdot g_a\ G_l$
2. $G_a\ g_l \cdot g_a\ g_l \times G_a\ G_l \cdot g_a\ G_l$
3. $g_a\ g_l \cdot g_a\ g_l \times G_a\ G_l \cdot G_a\ G_l$

The first combination gives only one type of F_1 plants, $G_a\ g_l \cdot g_a\ G_l$, all of which must produce excess of recessives. But this is not the case. The second combination, as a result of crossing over, produces four types of F_1 plants, namely:

F_1 genotypes	*Behavior in F_2 grains*
(A) $G_a g_l \cdot G_a G_l$	Normal recessive
(B) $G_a g_l \cdot g_a G_l$	Excess of recessive
(C) $g_a g_l \cdot g_a G_l$	Normal recessive
(D) $g_a g_l \cdot G_a G_l$	Deficiency of recessive

From the fifty-eight F_1 plants and their F_2 progenies studied, no positive evidence was obtained for the behavior required by the genotype $G_a\ g_l \cdot g_a\ G_l$. Some F_1 plants were obtained, for example, 103205B, which produced 21.96 percent glutinous grains in its first crop and 30.72 percent recessives in its sixth crop. This phenomenon of producing low recessives at one time and high recessives at another by the same plant without germinal change cannot be explained by the hypothesis of MANGELSDORF and JONES. The third combination calls for three types of progenies in F_3, namely, high recessive, normal recessive and low recessive. An examination of ninety-six F_3 segregating progenies showed two progenies giving high recessive, another two giving low recessive, and ninety-two producing normal recessive. While these four progenies may be cited in favor of the hypothesis, they may be attributed to chance equally well, unless further data point to the contrary.

It may be further noted that the F_2 progenies of KEMPTON (1919) gave an average of 23.7 percent waxy, while his F_3 progenies, an average of 24.6 percent waxy. It is the difference between these two generations that leads MANGELSDORF and JONES to postulate their hypothesis of an accessory factor linked with the waxy gene, for they think that the difference can

not be attributed to the "unfavorable influences inherent to the waxy gene itself." This seems, however, to be unnecessary. If the *same rice plant*, for example 103205B, can produce 21.96 percent glutinous grains at one time and 30.72 percent recessive at another without its genotypic constitution being changed, why should we be alarmed when KEMPTON'S F_2 progenies in maize behave differently from his F_3 progenies grown under different environmental conditions over a range of several years! It is true that the difference between the two generations of KEMPTON in maize is 4.3 times the probable error and would be expected as the result of chance only once in about 267 trials. But it is equally true that the difference in the two crops of the same plant (103205B) in rice is, as shown in table 17, 4.74 times the probable error and would be expected as the result of chance only once in about 655 trials. In brief, the results of the F_2 and F_3 generations in rice lend no support to the hypothesis of an accessory factor. In maize, where MANGELSDORF and JONES have reclassified KEMPTON'S results into high recessive, normal recessive, and low recessive groups, the evidence for their conclusion is meagre, and their usage of a deviation two times the probable error as a criterion is of doubtful value.

TABLE 17

The distribution of waxy and non-waxy maize seed in F_2 and F_3 (KEMPTON'S data) as compared with the proportion of glutinous and non-glutinous seed found in the first and sixth crops from a single rice plant (103205B).

	TOTAL SEEDS	RECESSIVE SEEDS	EXPECTED RECESSIVES	DEVIATION	DEV. / P.E.	PERCENT RECESSIVE	PERCENT DIFFERENCE / P.E. DIFFERENCE	ODDS
Maize F_2	77098	18267	19274	−1007	12.4	23.7	4.3	267.2:1
F_3	25329	6235	6332	− 97	2.09	24.6		
Rice 1st crop	783	172	195.75	− 23.75	2.90	21.96	4.74	655.3:1
6th crop	345	106	86.25	19.75	3.64	30.72		

In the writer's opinion, the distorted ratio in rice may very well be attributed to the disturbing effect of the glutinous gene itself. The most vigorous vegetative activity of the male gametophyte is shown during pollen tube growth. If the glutinous gene, g_l, is in a sense weaker in its dynamic activity than its normal allelomorph G_l, it is conceivable that the pollen tube carrying the gene g_l will grow more slowly than the pollen tube carrying G_l. Since our deficiencies are so small in individual cases, any slight differential activity between the allelomorphs, *Gl gl*, will produce the

observed deviations. That these small apparently insignificant deviations lead to a significant deficiency in the long run is also indubitable. The physiological activity of the gene depends in part on its environment. Under one condition, it acts one way and under another condition, it may act differently. This is an observed phenomenon in *Drosophila melanogaster*. The abnormal abdomen character is a case in point. Although the pollen tube carrying the glutinous gene grows more slowly under certain physiological conditions, it may grow faster under another set of environmental factors. This is in full accordance with the experimental data in the case of the F_1 plant, 103205B, which produced 21.96 percent glutinous grains in the first crop and 30.72 percent in the sixth crop. Observations show that excessively high temperature and low humidity contribute to the so-called seasonal infertility. Experimental data indicate that as seasonal infertility increases, the percentage of non-glutinous grains in F_2 decreases in favor of the glutinous type. It is possible that whatever environmental factors, such as temperature and humidity, are unfavorable for the activity of the non-glutinous gene on the one hand, may be favorable for the functioning of the glutinous gene on the other hand. This seems to be clearly indicated by the parallel increase in percentage of glutinous grains "seasonal infertility". In the light of all these facts, it appears justifiable to attribute the distorted glutinous ratio to the disturbing influences inherent in the glutinous gene itself. How the glutinous gene operates in causing different rate of pollen tube growth is an open question. In this connection, it is essential to bear in in mind that rice is an hermaphroditic seed plant which has an alternation of generations. Pollen tube development intervenes between the reduction divisions and fertilization. Here we are dealing with a delicate situation where the problem of heredity and the problem of development are intimately related. BRINK (1925) has well said, "When the 'latitude of genetic indetermination' is relatively wide, our problem becomes as much a physiological as a genetic one."

While the problems of heredity are being worked out satisfactorily, studies concerning the mechanics of development are just beginning. Preliminary study of the composition of glutinous and non-glutinous starch prepared in the pure form from the endosperm of the two types of rice, has shown that the glutinous starch contains about 12.72 percent crude α-amylose and 83.55 percent β-amylose, whereas the non-glutinous starch contains 75.48 percent α-amylose and 17.02 percent β-amylose. It is hoped that through the study of enzyme activity in pedigreed cultures of non-glutinous and glutinous plants in the future, we may learn

more about the physiology of the glutinous gene and its disturbing effect on pollen tube development.

SUMMARY

1. The non-glutinous and glutinous pair of characters is inherited in the Mendelian manner, non-glutinous being apparently completely dominant.

2. Abundant data have been brought forward to prove that they are due to a single pair of genetic factors which are designated as G_l g_l. In an F_2 population of 57,925 individuals, the ratio of non-glutinous grains is 3.04:0.96. YAMAGUCHI's dihybrid hypothesis is considered invalid.

3. It has been demonstrated beyond reasonable doubt that there is a significant deficiency of the recessive type as the result of self-pollinating heterozygous plants.

4. On the basis of a 3:1 ratio, the deviation from the expected percentage of glutinous grains in the total F_2 population of fourteen combinations is 8.52 times the probable error, and would be expected as the result of chance alone only once in about fourteen million trails.

5. Sufficient data have shown also that the significant deficiency in the totals is due to the accumulation of many smaller deviations in the same direction, each of which may be so small in the individual case as to escape notice.

6. F_3 tests have shown that the deficiency is not caused by wrong classification.

7. Pollen counts have demonstrated that segregating plants produce non-glutinous and glutinous pollen grains in equal numbers, showing that the deficiency of the recessive class is not due to any irregularities at sporogenesis.

8. That the same heterozygous plant which produces a deficiency at one time may produce an excess at another time makes it improbable that selective fertilization or gametic incompatibility is the cause of the deficiency of glutinous grains.

9. Nor can the deficiency be attributed to seasonal infertility, because experimental evidence is brought forward showing that as seasonal infertility increases the proportion of glutinous grains produced also increases.

10. The fact that the spikelets are either completely filled or entirely empty without intermediate types of defective grains is taken to indicate that the deficiency is not occasioned by zygotic lethals.

11. By tracing through the life cycle of the plant, it is shown that the cause of the deficiency is probably to be sought during the development of the male gametophyte. It is postulated that the exact stage is during the vegetative growth of the pollen tube.

12. An accessory factor hypothesis similar to that MANGELSDORF and JONES have advanced to account for the waxy deficiency in maize is not applicable to the present case in rice.

13. The primary cause of the differential pollen tube development which occasions the distorted non-glutinous and glutinous ratio is attributed to the influences inherent to the glutinous gene itself.

14. Experimental evidence has been brought forward indicating that pollen tubes carrying the glutinous gene and growing slower under ordinary conditions, may grow faster under particularly favorable conditions such as temperature and humidity, thus producing an excess of recessives.

15. On the assumption that environmental factors can influence the effect of the glutinous gene on the rate of pollen tube development, the discrepancies observed by earlier investigators may be accounted for.

ACKNOWLEDGMENT

The author is indebted to Dr. R. A. BRINK for his valuable suggestions in the progress of the work and kind criticisms in the preparation of the paper.

LITERATURE CITED

BRINK, R. A., 1925 Mendelian ratios and the gametophyte generation in angiosperms. Genetics **10:** 359–394. *2 figs.*

1927 The sugary gene in maize as a modifier of the waxy ratio. Genetics **12:** 461-491.

BRINK, R. A., and ABEGG, F. A., 1926 Dynamics of the waxy gene in maize. I. The carbohydrate reserves in endosperm and pollen. Genetics **11:** 163–199. *9 figs. 1 col. pl.*

BRINK, R. A., and MACGILLIVRAY, J. H., 1924 Segregation for the waxy character in maize pollen and differential development of the male gametophyte. Amer. Jour. Bot. **11:** 465–469.

CHANG, C. C., 1921 Rice inheritance. Jour. Agric. Assoc. of China, Rice No. 21–30.

COLLINS, G. N., 1909 A new type of Indian corn from China. U. S. Dept. Agric. Bureau of Plant Industry Bull. 161: 1–30.

COLLINS, G. N., and KEMPTON, J. H., 1911 Inheritance of waxy endosperm in hybrids of Chinese maize. 4th Confer. Inter. Génét., Paris: 347–356.

COPELAND, R. B., 1924 Rice. First edition. pp. 119–120. New York: Macmillan Co.

CZAPEK, F., 1913 Biochemie der Pflanzen. Band **1:** 1–828.

DAFERT and MEYER (See TANAKA 1907)

DEMEREC, M., 1924 A case of pollen dimorphism in maize. Amer. Jour. Bot. **11:** 461–464.

GRIS, A., 1860 Note sur la fécule du riz. Bull. de la soc. Bot. France **7:** 876–877.

HOSHINO, Y., 1902 Xenia in the crosses between *Oryza glutinosa*×*O. sativa*. Jour. Sapporo Agric. Soc. **2:** 90–92.

IKENO, S., 1914 Ueber die Bestäubung und die Bastardierung von Reis. Zeitschr. f. Pflanzenzücht. **2:** 495–503.

KEMPTON, J. H., 1919 Inheritance of waxy endosperm in maize. U. S. Dept. Agric. Bureau of Plant Industry Bull. 754: 1–99.

1921 Waxy endosperm in Coix and Sorghum. Jour. Heredity **12:** 396–400.

KIESSELBACH, T. A., and PETERSEN, N. F.,1926 The segregation of carbohydrates in crosses between waxy and starchy types of maize. Genetics **11:** 407–422.

LINDSTROM, E. W., and GERHARDT, F., 1926 Inheritance of carbohydrates and fat in crosses of dent and sweet corn. Iowa Agric. Exp. Sta. Res. Bull. 98: 259–277.

LONGLEY, A. E., 1924 Chromosomes in maize and maize relatives. Jour. Agric. Res. **28:** 673–682.

MANGELSDORF, P. C., and JONES, D. F., 1926 The expression of Mendelian factors in the gametophyte of maize. Genetics **11:** 423–435.

MEYER, ARTHUR, 1886 Ueber Stärkekörner welche sich mit Jod farben. Bericht. Deutsch. Bot. Gesell. **4:** 337–362.

PARNELL, F. R., 1921 Note on detection of segregation by examination of pollen of rice. Jour. Genetics **11:** 209–212.

SHARNGAPANI, S. G., 1924 A few observations on paddy crossing. Agro. Jour. India **1:** 48–50.

TADOKORO, T., NAKAMURA, Y., and WATANABE, S., 1926 On the differences between some colloidal and chemical properties of common and glutinous rice starch. II. Ibid. **16:** 73–123.

TADOKORO, T., and SATO, S., 1923 On the differences between some colloidal properties of common and glutinous rice starch. Jour. Coll. Agric. Hokkaido Imp. Univ. Sapporo, Japan **13:** 1–65.

TANAKA, Y., 1907 On the starch of glutinous rice and its hydrolysis by diastase. Jour. Coll. Eng. Tokyo Imp. Univ. 4, No. 2.

1912 On the starch of glutinous rice and its hydrolysis by diastase. Jour. Indust. and Eng. Chemistry 4, No. 8.

YAMAGUCHI, Y., 1926 Genetische analyse der Granne, der Spelzenfarbe und der Endospermbeschaffenheit bei einigen Sorten des Reises. Berichte des Ohara Instituts für Landwirtschaftliche Forschungen, Bd. III, Heft 1: 1–126.

APPENDIX

TABLE 16

Ninety-seven F_3 progenies from the cross 4269×4957 segregating for the glutinous character.

PLANT NUMBER	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEVIATION / P. E.	PERCENT GLUTINOUS
Progenies with excess over 3 times the probable error						
EST 47	245	105	350	17.5	3.20	30.0
FST 38	349	143	492	20.0	3.08	29.07
Sub-total	594	248	842	37.5	4.42	..
Progenies with deficiency over 3 times the probable error						
AST 33	343	89	432	−19	3.13	20.60
CST 32	114	12	126	−19.5	5.94	9.52
IST 40	226	52	278	−17.5	3.59	18.71
Sub-total	683	153	836	56.0	6.63	..
Progenies with no significant deviation						
AST 13	197	74	271	6.25	1.29	27.31
AST 20	298	107	405	5.75	0.97	26.42
AST 28	656	215	871	− 2.75	0.31	24.68
AST 34	255	97	352	9.0	1.64	27.56
AST 35	170	48	218	− 6.5	1.50	22.02
BST 18	201	72	273	3.75	0.77	26.38
BST 20	284	82	366	− 9.5	1.69	22.40
BST 21	276	86	362	− 4.5	0.80	23.76
BST 22	315	109	424	3.0	0.49	25.71
BST 26	193	62	255	− 1.75	0.37	24.31
BST 30	270	75	345	−11.25	2.07	21.74
BST 39	158	56	214	2.5	0.58	26.17
BST 43	243	79	322	− 1.5	0.28	24.53
CST 1	110	38	148	1.0	0.28	25.68
CST 6	155	51	206	− 0.5	0.11	24.75
CST 11	87	27	114	− 1.5	0.48	23.68
CST 17	95	35	130	2.5	0.75	26.92
CST 22	167	53	220	− 2	0.46	24.09
CST 23	120	35	155	− 3.75	1.03	22.58
CST 24	122	39	161	− 1.25	0.33	24.22
CST 25	156	47	203	− 3.75	0.9	23.15
CST 43	168	56	224	0	..	25.00
CST 51	225	74	299	− 0.75	0.14	24.75
CST 52	125	37	162	− 3.5	0.94	22.84
CST 53	237	72	309	− 5.25	1.04	23.30
CST 56	147	49	196	0	..	25.00
CST 60	223	70	293	− 3.25	00.65	23.89
DST 16	262	88	350	0.5	0.09	25.14
DST 18	190	50	240	−10.0	2.21	20.83

TABLE 16 (continued)

PLANT NUMBER	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEVIATION / P. E.	PERCENT GLUTINOUS
Progenies with no significant deviation						
DST 32	231	87	318	7.5	1.43	27.36
DST 33	315	99	414	− 4.5	0.76	23.91
DST 38	209	67	276	− 2.0	0.41	24.27
DST 39	483	136	619	− 8.75	1.20	21.97
DST 40	272	93	365	1.75	0.31	25.48
DST 41	233	81	314	2.5	0.48	25.80
DST 56	275	77	352	−11.0	2.00	21.88
DST 57	255	88	343	2.25	0.41	25.65
DST 59	280	89	369	− 3.25	0.57	24.12
DST 62	177	54	231	− 3.75	0.84	23.38
EST 2	238	91	329	8.75	1.65	27.66
EST 16	224	67	291	− 5.75	1.16	23.02
EST 17	150	63	213	9.75	2.28	29.58
EST 19	250	83	333	− 0.25	0.04	24.92
EST 26	168	45	213	− 8.25	1.93	21.12
EST 28	193	69	262	3.5	0.74	26.34
EST 36	240	101	341	15.75	2.92	29.62
EST 43	268	71	339	−13.75	2.55	20.94
EST 45	161	55	216	1.0	0.23	25.46
EST 46	221	58	279	−11.75	2.40	20.79
FST 8	216	70	286	− 1.5	0.3	24.47
FST 14	196	64	260	− 1.0	0.21	24.61
FST 17	172	49	221	− 6.25	1.2	22.17
FST 21	197	68	265	1.75	0.36	25.66
FST 23	333	91	424	−15.0	2.49	21.46
FST 42	158	55	213	1.75	0.41	25.82
FST 44	129	40	169	− 2.25	0.59	23.67
FST 48	172	47	219	− 7.75	1.79	21.46
FST 49	432	125	557	−14.25	2.06	22.44
FST 51	209	84	293	10.75	2.15	28.67
FST 58	105	35	140	0	..	25.00
FST 64	249	86	335	2.25	0.42	25.67
FST 65	152	55	207	3.25	0.77	26.57
GST 7	198	67	265	0.75	0.15	25.28
GST 9	218	76	294	2.5	0.49	25.85
GST 11	192	50	242	−10.5	2.31	20.66
GST 21	257	90	347	3.25	0.59	25.94
GST 36	220	58	278	−11.5	2.36	20.86
GST 45	241	76	317	− 3.25	0.62	23.97
GST 48	153	68	221	12.75	2.93	30.77
GST 49	194	72	266	5.6	1.17	27.07
IST 9	236	82	318	2.5	0.48	25.75
IST 10	240	76	316	− 3.0	0.57	24.05

TABLE 16 (continued)

PLANT NUMBER	NON-GLUTINOUS GRAINS	GLUTINOUS GRAINS	TOTAL	DEVIATION	DEVIATION / P. E.	PERCENT GLUTINOUS
Progenies with no significant deviation						
IST 13	271	92	363	1.25	0.22	25.34
IST 15	199	51	250	−11.5	2.49	20.40
IST 17	248	81	329	− 1.25	0.23	24.62
IST 23	300	86	386	−10.5	1.84	22.28
IST 33	279	83	362	− 7.5	1.34	22.93
IST 49	246	76	322	− 4.5	0.85	23.6
IST 60	237	76	313	2.25	0.43	24.28
IST 74	359	121	480	1.0	0.15	25.21
JST 4	201	67	268	0	0	25.00
JST 25	313	124	437	14.75	2.41	28.37
JST 35	248	80	328	− 2.0	0.37	24.40
JST 43	204	75	279	5.25	1.07	26.88
JST 59	232	59	291	−13.75	2.76	20.27
JST 62	199	73	272	5.0	1.03	26.84
JST 64	256	86	342	− 0.5	0.09	25.14
JST 69	157	40	197	− 9.25	2.25	20.30
JST 73	331	103	434	− 3.0	0.49	23.73
JST 82	232	73	305	− 3.25	0.63	23.93
Sub-total	20329	6587	26916	−142.0	2.96	24.47
Grand total	21606	6988	28594	−160.5	3.23	24.44

BIONOMIC STUDIES ON CERTAIN TELEOSTS (Poeciliinae). II. COLOR PATTERN INHERITANCE AND SEX IN *Platypoecilus maculatus* (Günth.)

A. W. BELLAMY

University of California at Los Angeles, California

Received October 21, 1927

The fishes upon which the accompanying observations were made were described in Study I of this series (BELLAMY 1924). Also a preliminary account of the hereditary behavior displayed by them was given at the Toronto meetings of the American Society of Zoologists, 1922 (See Anatomical Record, 1924, pp 419–420). Since that time enough data have accumulated to make it reasonably certain that the interpretation previously indicated is the correct one, namely, that the color varieties named Nigra (*N*), Pulchra (*P*), Rubra (*R*), and White (*W*) constitute a multiple allelomorphic series of sex-linked characters, with the female heterogametic for sex factors. Males may be heterozygous for any two but not more than two of the factors for these characters. Females carry only one. Hence males may be described as *NN*, *NP*, *NR*, *NW*, *PP*, *PR*, *PW*, *RR*, *RW*, and *WW*; females as *NO*, *PO*, *RO*, and *WO*.

In the accompanying pedigree the animals are represented by these symbols and while they serve to indicate the interpretation just mentioned, since they also describe the color and sex of the fishes so recorded, the data can be compared with any hypothesis the reader may have in mind.

GORDON (1927) whose paper came to my attention some time after this report was in manuscript, objects to certain of the above statements. He suggests "stippled" as a more suitable name for "White." His term more accurately describes the (microscopic) pattern of the fish but not the general appearance of the animal as one sees it with the unaided eye.

While I did not consider a factor for stippled under that name the existence of the small melanophores characteristic of the white platypoecilus was not ignored (BELLAMY 1924, page 523). So far as the description of hereditary behavior in the four varieties here described is concerned either term may be used.

GORDON seems to think that the factors *W*, *N*, *P*, *R*, do not constitute a multiple allelomorphic series, meaning, I suppose, that *W* does not belong in the series. Perhaps it does not. The data presented must speak for themselves.

Objection is raised to the statement that "males may be heterozygous for any two of the four characters, *W*, *P*, *R*, or *N*; and the female may carry the determiners for only one". So far as my experience goes the statement is true as it stands, and if one may resort to what approaches hear-say evidence, so far as I am aware none of the numerous Chicago fanciers ever obtained a Rubra-Pulchra, Rubra-Nigra, or Nigra-Pulchra

TABLE 1

White male × Colored female	
WW	*CO*

Colored males	White females
CW	*WO*
F_1 127	116

F_2 Colored males	White males	Colored females	White females
CW	*WW*	*CO*	*WO*
46	74	70	83

female, although to my knowledge they attempted it repeatedly. Further it is not clear from GORDON's data (his table 2 and pages 268–270) that he had a pulchra-rubra female.

TABLE 2

Colored male × White female	
CC	*WO*

Colored males	Colored females
CW	*CO*
347	358

Colored males		Colored females	White females
CC	*CW*	*CO*	*WO*
411		289	258

Crosses involving Golden and Rubra as well as Nigra, Pulchra and White were made in 1923–24 and further discussion of GORDON's data can be undertaken more profitably in connection with my own data on the same types of crosses.

It will be sufficient here to add that White (stippled) whether sex-linked or not is used in exactly the same sense as "wild-type" in Drosophila.

There is not the slightest difficulty in separating the four color varieties from one another but certain of the heterozygous males are not readily distinguished. *PP* and *PR* look much alike until they are fully

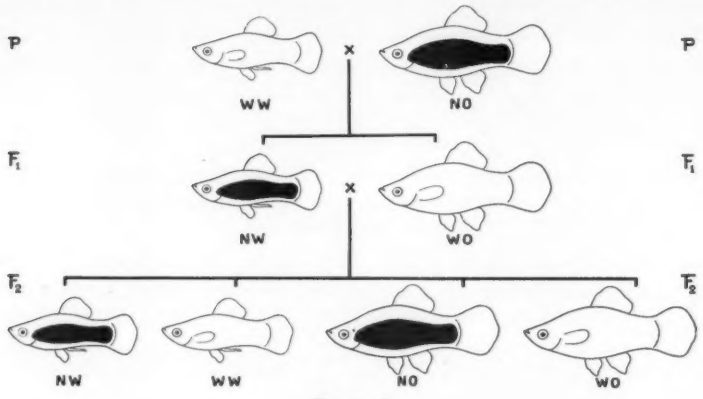


FIGURE 1.

mature when the red color and heavier pigmentation of *PR* enables one to distinguish most of them. In progenies where both *NP* and *PP* or *PR* appear there are usually a few males that are not readily distinguish-

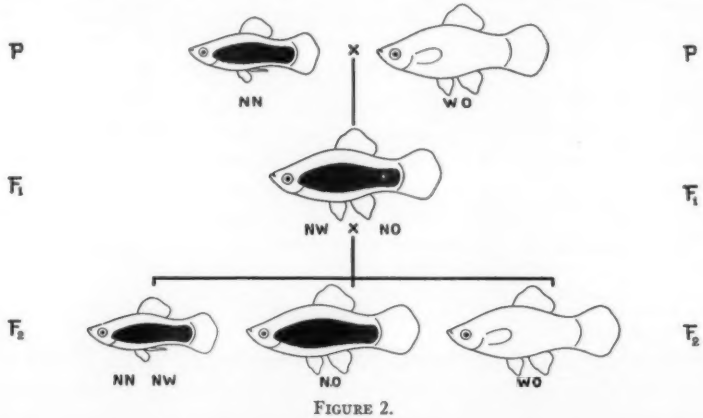


FIGURE 2.

able. Also since white is recessive to all of the other color characters, *NW*, *PW*, and *RW* must be tested by breeding. Obviously, however, in pedigree material the type of mating enables one to know whether the males

will be heterozygous for *W*. For the most part we avoid those matings that give combinations difficult to separate accurately.

As far as possible all of the relevant information concerning the hereditary behavior of these four color varieties is included in the accompanying pedigree. In the first column which describes the mating, males are indicated on the left of the ×. In several cases the same male is used in more than one mating. This is indicated by a sub-numeral following the descriptive formula of the male, the same sub-numeral being used in all of the matings in which that particular male appears. The NN_3 male in mating 261 is the same individual that appears in mating 31.2.3; the male NR_9 is used in matings 273, 272, and 217.2. The second column in the pedigree gives the original record number, and in the third and fourth columns the source of the male and female parent is indicated. The number of young born in each mating is given in the fifth column and in the following 18 columns the color and sex of the offspring recorded for each mating. In the two last columns the number and percentage of offspring not recorded is given.

No evidence of crossing over has yet come to light so that unless the four characters are multiple allelomorphs, as we assume, they must be very closely linked. At any rate the type of hereditary behavior can be seen to good advantage by grouping the matings according to the scheme used in tables 1 and 2—which represent the F_1 and F_2 generations of reciprocal crosses involving a sex-linked characters. (See also figures 1, 2). *NN*, *NW*, *NO*, *PP*, *PW*, *PO*, *RR*, *RW*, *RO*, *NP*, *PR*, *NR*, are classified as "colored". A white male when crossed with a colored female produces colored sons and white daughters. Combining the results of mating 25, 262, 263, 268, and 291 we have in F_1 127 colored sons and 116 white daughters; in F_2 (matings 83, 263.1, 268.1, 314, and 327) 46 colored and 74 white males, 70 colored and 83 white females (table 1). The reciprocal crosses (table 2) give in F_1 all colored offspring—347 males and 358 females (matings 31, 217.2, 255, 259, 265, 286, 289, 290, 298, and 316). In F_2 (matings 21, 21.1, 21.2, 31.1, 31.2, 44, 44.2, 44.2.1, 82, 83.1, 83.2, 89, 89.1, 89.1.1, 217, 217.2.1, 217.2.2, 217.2.3, 259.1, 269.2, 265.1, and 289.1) there are 411 colored males, 289 colored females and 258 white females. In addition there appeared 4 white males, 3 in mating 44.2.1 and 1 in mating 89.

The three white males (44.2.1) appeared in the first brood of 39 young and were isolated as males when 128, 149, and 157 days old. The white male in 89 was about 8 months old when isolated.

It is likely that these 4 males were in some way introduced into the tanks by accident. Although we exercise great care to prevent contamination it sometimes occurs. For example matings 314 and 327 were made to test males apparently out of place where they were first discovered. The male *PW* tested in mating 314 was found in the third brood of young from mating 258. It happened that the second brood of mating 263 was born on the same day. Since these were the only other young born near the date of birth of the *PW* male and since some one in the laboratory, about this time adopted a large bulb pipette for counting the new born young and transferring them to a new tank I feel justified, with this explanation, in including this *PW* male in the records of mating 263 instead of 258. The male tested in mating 327 was found in the third brood of young from mating 272. It is likely that he belongs with mating 268, young from which were born the same day. Needless to say the use of hot water for rinsing the pipette was strictly enforced after these two experiences.

While it is probable that the 4 white males found in the progenies of matings 44.2.1 and 89 are the result of contamination it is possible that they represent crossovers of some sort. Possibly they are a consequence of non-disjunction in the mothers, although one would infer from the heterogametic nature of females—if one may infer at all that the fertilization of a non-disjunctional gamete would result in a female. Perhaps these males represent an inversion of sex in some of the white females, an occurrence for which there is some circumstantial, but no direct, evidence.[1]

In the two matings, 44.2 and 83.1, there is some departure from expected ratios. In the former the male is regarded as heterozygous for the reason that one of his sons, the one mated in 44.2.1 was certainly heterozygous. However the number of recorded young is so small that little significance attaches to the absence of one expected phenotype. But in mating 83.1 where the male must be heterozygous because of his ancestry, it is curious that among the 26 recorded young there should have been no white females. The case is made somewhat more puzzling by the fact that all of the sons of the 83.1 male that were tested proved to be homozygous. It will be seen that a brother of this male (mating 83.2) was heterozygous.

[1] ESSENBERG (1926) has described "complete sex-reversal" in a closely related species, *Xiphophorus helleri*. Two individuals are said to have given birth to young as females and later to have been the fathers of young. It is highly desirable that these very interesting observations be repeated, under circumstances where they can be kept under continuous observation. (See page 99 of ESSENBERG's paper).

With reference to the possibility of the male in 83.1 being a result of contamination it should be said that until the offspring from matings 82 and 83 were born the only pulchras in the entire laboratory were the two males used as fathers in these two matings. By assuming crossing over in the male *PW* used in 83 or even in his son, used in 83.1 and invoking the aid of homozygous lethals it is not particularly difficult to make a restatement in substantial agreement with the absence of white females in 83.1. If, for instance, we assume that instead of being allelomorphs *P* and *W* are very closely linked and that the male used in 83.1 resulted from a cross over gamete in the 83 male, then the 83.1 male would have genes for both *P* and *W* in one X chromosome, the other X chromosome being without genes for these characters and therefore, let us say, giving rise to a nonviable gamete, or perhaps, when fertilizing a *W*-carrying gamete producing a non-viable zygote. If all this is true then a number of the males in the "83" line of descent are of the composition (*PW*)*X*-(*P*)X and the females (*PW*)X-Y. I have no real evidence bearing on the matter and until another case appears speculation does not appear to be particularly fruitful.

Attention should be called to mating 89. This female was originally mated to an F_2 hybrid male from a cross of *Xiphophorus helleri* male *Platypoecilus maculatus* (*nigra*) female. The record bears the notation "female may not be virgin" a statement that is borne out by the kind of of young she produced. It is highly probable that the male parent of the young in this mating is a *NW* brother of the female the only type of male, other than the hybrid mentioned, to which she could have been exposed. The mating appears in the pedigree as though the *NW* male had been used in the original mating.

The female used in mating 100 was without an anal fin. She was discovered when about 4 weeks old. All of the F_1 and F_2 young were normal.

In mating 261 the first male, *NN* from 31.2, died 27 days after the mating was made. Fifty-four days later a new male *NN* from 31.2.6.2 was added. The first young were born 71 days after the introduction of the second male. Undoubtedly he is the father of the young in mating 261.

While the inheritance of color pattern in Platypoecilus is clearly of the poultry-type there are no data available for profitable discussion of any possible or probable association between the characters described and sex chromosomes. It would be the obvious thing to predict a ZZ, WZ chromosome equipment.

AIDA (1921) describes a type of sex-modified hereditary behavior in *Aplocheilus latipes*, and oviparous species, very similar to that described

for *Lebestes reticulatus* by WINGE (1922). In both cases males are described as being heterogametic for sex factors and certain genes are associated with the Y-chromosome to account for a father-to-son type of inheritance. In *Lebestes* the problem is complicated by the fact that color pattern characters are strictly sex-limited, appearing only in males. In spite of the fact that it was not possible to establish any morphological differences between X and Y chromosomes in Lebestes and that, further, WINGE has not "succeeded in laying down any morphological or other differences in the chromosomes, which render it possible to distinguish the sex-chromosomes from the autosomes", he takes the position that his data "prove" the existence of X- and Y-chromosomes.

In the light of present knowledge it does not seem easy to construct a mechanism that will satisfy both the Lebestes—Aplocheilus and Platypoecilus types of sex-modified hereditary behavior. Nor for that matter is there any *a priori* reason for assuming that they need be identical. It is quite possible that, as in certain Lepidoptera among insects, these species of fishes, although fairly closely related, have evolved mechanisms of sex determination and inheritance that exhibit some detailed differences.

SUMMARY

A multiple allelomorphic series of four sex-linked color-pattern characters in *Platypoecilus maculatus* Günth. is described. The female is heterogametic for sex factors.

LITERATURE CITED

AIDA, TATUO, 1921 On the inheritance of color in a fresh-water fish, *Aplocheilus latipes* Temmick and Schlegel, with special reference to sex-linked inheritance. Genetics **6**: 554–573.

BELLAMY, A. W., 1922 Sex-linked inheritance in the teleost *Platypoecilus maculatus* Günth. Anat. Rec. **24**: 419–420.

1924 Bionomic studies on certain teleosts (poeciliinae). I. Statement of problems, description of material, and general notes on life histories and breeding behavior under laboratory conditions. Genetics **9**: 513–529.

ESSENBERG, J. M., 1926 Complete sex-reversal in the viviparous teleost *Xiphophorus helleri* Biol. Bull. **51**: 98–111.

GORDON, MYRON, 1927 The genetics of a viviparous top-minnow platypoecilus; the inheritance of two kinds of melanophores. Genetics **12**: 253–283.

WINGE, O., 1922 One-sided masculine and sex-linked inheritance in *Lebestes reticulatus*. Jour. Genetics **12**: 145–162.

TABLE 3
Pedigree

MATING	RECORD NUMBER	SOURCE OF PARENTS		YOUNG BORN	NN	NN NW	NW	NO	PP	PP PW	PW	PO	RR	RR RW	RW	RO	WW	WO	NP	NP PR	PR	NR	NOT RECORDED	PERCENT NOT RECORDED
		Male	Female																					
$RW_1 \times RO$	21	Unknown	Unknown	20	..	..	..	..	..	..	..	..	..	5	..	2	..	5	..	..	..	..	8	40
$RW_2 \times RO$	21.1	21	21	3	..	..	..	..	..	..	..	..	..	1	..	1	..	1	..	..	..	..	0	0
$RW_3 \times RO$	21.2	Unknown	21	139	..	..	..	..	..	..	..	..	..	28	..	45	..	35	..	..	..	..	31	22
$WW_2 \times PO$	25	Unknown	Unknown	2	..	..	..	..	..	..	2	..	..	..	..	..	..	0	..	..	..	..	0	0
$NN \times NO$	30	Unknown	Unknown	45	21	..	..	23	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	2
$NN \times WO$	31	Unknown	Unknown	48	..	..	9	6	..	..	..	..	..	..	..	..	..	..	..	..	..	..	33	69
$NW \times NO$	31.1	31	31	64	..	14	..	11	..	..	..	..	..	..	..	..	..	25	..	..	..	..	14	22
$NW \times NO$	31.2	31	31	87	..	26	..	18	..	..	..	..	..	..	..	..	..	21	..	..	..	..	22	25
$NN_2 \times NO$	31.2.3	31.2	31.2	61	25	..	..	29	..	..	..	..	..	..	..	..	..	..	..	..	..	..	7	11
$NN \times NO$	31.2.6	31.2	31.2	8	4	..	..	4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	0
$NN_4 \times NO$	31.2.6.2	31.2.6	31.2.6	85	39	..	..	36	..	..	..	..	..	..	..	..	..	..	..	..	..	..	10	12
$NN \times NO$	31.4.1	31.4	31.4	67	7	..	..	6	..	..	..	..	..	..	..	..	..	..	..	..	..	..	54	81
$WW_2 \times WO$	35	Unknown	Unknown	6	..	..	..	..	..	..	..	..	..	..	..	..	..	2	3	..	..	..	1	17
$RW \times RO$	44	Unknown	Unknown	80	..	..	..	..	..	..	..	..	..	25	..	23	..	7	..	..	..	..	25	31
$RR \times RO$	44.1	44	44	232	..	..	..	..	..	..	..	..	35	..	..	69	..	..	..	..	..	..	128	55
$RW \times RO$	44.2	44	44	20	..	..	..	..	..	..	..	..	..	3	..	7	..	0	..	..	..	..	10	50
$RW \times RO$	44.2.1	44.2	44.2	87	..	..	..	..	..	..	..	..	12	..	16	3	11	..	..	..	..	..	45	52
$RR \times RO$	44.3	44.1	44.1	75	..	..	..	..	..	..	..	..	..	15	..	23	..	..	..	..	..	..	37	51
$RR_3 \times RO$	44.3.2	44.3	44.3	54	..	..	..	..	..	..	..	..	..	18	..	29	..	..	..	..	..	..	7	13
$RR \times RO$	44.4	44.1	44.1	10	..	..	..	..	..	..	..	..	..	5	..	5	..	..	..	..	..	..	0	0
$WW_3 \times WO$	46	Unknown	Unknown	40	..	..	..	..	..	..	..	..	..	..	..	..	16	14	..	..	..	..	10	25
$RR \times RO$	74	Unknown	Unknown	30	..	..	..	..	..	..	..	..	12	..	..	12	..	..	..	..	..	..	6	20
$PW \times NO$	82	25	30	31	..	..	9	..	..	..	..	7	..	..	..	..	..	6	5	..	..	..	4	13
$PW \times WO$	83	25	35	38	..	..	..	..	..	..	7	9	..	..	..	..	5	17	..	..	..	..	0	0
$PW \times PO$	83.1	83	83	34	..	..	..	..	..	15	..	11	..	..	..	..	..	0	..	..	..	..	8	23
$PW \times PO$	83.2	83	83	184	..	..	..	..	45	..	..	21	..	..	..	..	..	19	..	..	..	..	99	54
$PP_4 \times PO$	83.1.2	83.1	83.1	83	..	..	..	36	..	..	..	35	..	..	..	..	..	..	..	..	..	..	12	14
$PP \times PO$	83.1.3	83.1	83.1	59	..	..	..	27	..	..	..	22	..	..	..	..	..	..	..	..	..	..	10	17
$PP \times PO$	83.1.3.1	83.1.3	83.1.3	90	..	..	..	13	..	..	..	12	..	..	..	..	..	..	..	..	..	..	65	72
$NW \times NO$	89	31	31	92	..	24	..	19	..	..	..	..	..	..	..	..	..	1	13	..	..	..	35	38
$NW \times NO$	89.1	89	89	37	..	13	..	6	..	..	..	..	..	..	..	..	..	..	6	..	..	..	12	32
$NW \times NO$	89.1.1	89.1	89.1	85	..	32	..	19	..	..	..	..	..	..	..	..	..	..	22	..	..	..	12	14
$WW \times WO$	100	83	83	24	..	..	..	..	..	..	..	..	..	..	..	..	..	2	7	..	..	..	15	63
$WW \times WO$	117	46	31.1	47	..	..	..	..	..	..	..	..	..	..	..	..	..	17	18	..	..	..	12	26
$WW \times WO$	117.1	117	117	92	..	..	..	..	..	..	..	..	..	..	..	..	..	8	4	..	..	..	80	87
$WW \times WO$	117.1.1	117.1	117.1	104	..	..	..	..	..	..	..	..	..	..	..	..	..	13	4	..	..	..	87	84
$WW \times WO$	188	46	31.1	131	..	..	..	..	..	..	..	..	..	..	..	..	..	30	54	..	..	..	47	36
$WW \times WO$	188.1	188	188	97	..	..	..	..	..	..	..	..	..	..	..	..	..	32	39	..	..	..	26	27
$WW_3 \times WO$	188.1.1	188.1	188.1	54	..	..	..	..	..	..	..	..	..	..	..	..	..	15	24	..	..	..	15	28
$WW_4 \times WO$	188.1.2	188.1	188.1	12	..	..	..	..	..	..	..	..	..	..	..	..	..	9	3	..	..	..	0	0
$RW \times NO$	217	21.2	89.1	18	..	..	..	5	..	..	..	..	..	..	..	..	7	..	4	..	..	2	0	0
Group	217.1	217	217	36	..	..	..	2	7	..	..	..	..	12	..	6	..	5	..	..	..	4	0	0
$NR_3 \times WO$	217.2	217	217	126	..	..	22	21	..	..	..	..	..	..	24	32	..	..	..	..	..	..	27	21
$NW \times RO$	217.2.1	217.2	217.2	164	..	..	..	29	..	..	..	..	..	..	30	..	..	27	..	..	..	11	67	41
$RW \times NO$	217.2.2	217.2	217.2	93	..	14	..	..	..	..	..	..	..	..	..	20	..	17	..	..	..	24	18	19
$NW \times RO$	217.2.3	217.2	217.2	88	..	..	..	17	..	..	..	..	..	..	22	..	..	25	..	..	..	16	8	9
$NN \times RO$	254	31.1	21	27	..	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	2	23	85
$NR_{10} \times NO$	254.1	254	254	120	11	..	..	27	..	..	..	..	..	..	..	21	..	..	..	..	..	20	41	34
$NN \times RO$	254.1.1	254.1	254.1	33	..	..	..	8	..	..	..	..	..	..	..	..	..	..	..	..	..	12	13	39
$NR_{10} \times WO$	255	254	188	113	..	..	14	27	..	..	..	..	..	..	20	21	..	..	..	..	..	..	31	27
$RR_{11} \times NO$	257	44.3	31.41	123	..	..	..	..	..	..	..	..	..	..	..	45	..	..	..	..	..	67	11	9
$RR_{12} \times NO$	258	44.3	89.1	144	..	..	..	..	..	..	..	..	..	..	..	56	..	..	..	..	..	54	34	24
$NR \times RO$	258.1	258	258	143	..	..	..	16	..	..	..	..	9	..	..	29	..	..	..	..	..	20	69	48
$RR \times WO$	259	44.3	188	195	..	..	..	..	..	..	..	..	..	..	67	..	..	71	..	..	..	..	57	29
$RW \times RO$	259.1	259	259	32	..	..	..	..	..	..	..	..	..	4	..	2	..	3	..	..	..	..	23	72
$RW \times RO$	259.2	259	259	22	..	..	..	..	..	..	..	..	..	1	..	2	..	4	..	..	..	..	15	68
$NN_2 \times PO$	261	31.2	83.1.2	40	..	..	..	20	..	..	..	..	..	..	..	..	..	..	11	..	..	..	9	22
$WW_3 \times NO$	262	188.1	31.2.3	95	..	..	43	..	..	..	..	..	..	..	..	..	..	47	..	..	..	..	5	5
$WW_3 \times PO$	263	188.1	83.1.2	74	..	..	..	..	..	..	36	..	..	..	..	..	..	30	..	..	..	..	8	11
$PW \times WO$	263.1	263	263	101	..	..	..	..	..	..	14	23	..	..	..	..	26	12	..	..	..	..	26	26
$PP_4 \times NO$	264	83.1	31.2.3	131	..	..	..	..	..	..	..	57	..	..	..	..	..	..	48	..	..	..	26	20
$NP \times PO$	264.1	264	264	98	..	..	..	11	9	..	..	15	..	..	..	..	..	..	11	..	..	..	52	53
$NN_4 \times WO$	265	31.2.6	188.1	145	..	..	73	51	..	..	..	..	..	..	..	..	..	..	..	..	..	..	21	14
$NW \times NO$	265.1	265	265	41	..	16	..	3	..	..	..	..	..	..	..	..	..	6	..	..	..	..	16	39
$RR \times PO$	266	Stock	83.1.2	141	..	..	..	..	..	..	..	..	..	..	..	40	..	..	..	..	37	..	64	45
$WW \times RO$	268	117.1	44.3	99	..	..	..	..	..	..	..	..	..	..	42	..	..	38	..	..	..	..	19	19
$WW \times WO$	268.1	268	268	26	..	..	..	..	..	..	..	..	..	..	3	1	4	4	..	..	..	..	14	54
$NR_{10} \times PO$	270	254	83.1.2	126	..	..	..	31	..	..	..	..	..	..	..	21	..	..	..	58	..	..	16	13
$PR \times RO$	270.1	270	270	54	..	..	..	..	..	..	..	8	7	..	..	12	..	..	..	..	8	..	19	35
$NR_{10} \times NO$	271	254	31.2.6.2	139	17	..	..	26	..	..	..	..	..	..	..	23	..	..	..	..	..	18	55	40
$NR_3 \times NO$	272	217	31.2.6.2	116	23	..	..	20	..	..	..	..	..	..	..	29	..	..	..	..	..	29	15	13
$NR_3 \times PO$	273	217	83.1.2	75	..	..	..	14	..	..	..	..	..	..	..	16	..	..	..	33	..	..	12	16
$RR_{11} \times WO$	286	44.3	117.1.1	71	..	..	..	..	..	..	..	..	..	..	32	27	..	..	..	..	..	..	12	17
$PP_4 \times WO$	289	83.1	117.1.1	88	..	..	..	..	..	..	38	30	..	..	..	..	..	..	..	..	..	..	20	23
$PW \times PO$	289.1	289	289	53	..	..	..	..	..	9	..	3	..	..	..	..	..	1	..	..	..	..	40	76
$NR \times WO$	290	257	117.1.1	56	..	..	5	3	..	..	..	..	..	..	5	15	..	..	..	..	..	..	28	50
$WW \times RO$	291	117.1.1	257	16	..	..	..	..	..	..	..	..	..	..	4	..	..	1	..	..	..	..	11	69
$RR_{12} \times WO$	298	44.3	117.1.1	64	..	..	..	..	..	..	..	..	..	..	13	18	..	..	..	..	..	..	33	52
$RR_3 \times RO$	308	44.3	44.3.2	99	..	..	..	..	..	..	..	..	22	..	..	27	..	..	..	..	..	..	50	50
$NN \times NO$	313	31.2.6.2	31.2.6.2	57	11	..	..	25	..	..	..	..	..	..	..	..	..	..	..	..	..	..	21	37
$PW \times WO$	314	263	268	112	..	..	..	..	..	..	7	14	..	..	..	..	18	21	..	..	..	..	52	46
$NP \times WO$	316	264	263	129	..	..	8	19	..	..	17	17	..	..	..	..	..	..	..	..	..	..	68	53
$PP \times PO$	320	83.1.3.1	83.1.3.1	57	..	..	..	..	17	..	..	10	..	..	..	..	..	..	..	..	..	..	30	53
$NN \times NO$	321	272	261	15	6	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	7	47
$RW \times WO$	327	268	268	172	..	..	..	..	..	..	..	..	..	15	23	21	29	..	..	..	..	..	84	49
				6524																			2238	

CYTOLOGICAL STUDIES IN THE GENUS NICOTIANA

M. CHRISTOFF[1]

Bussey Institution, Harvard University, Boston, Massachusetts

Received November 23, 1927

TABLE OF CONTENTS

Introduction 233
Method and technique 234
The Chromosomes of the various Nicotiana species 235
Hybridization results 243
Description of the species hybrids 246
Cytological observations on certain Nicotiana species hybrids 254
DISCUSSION 258
Inheritance in species hybrids 258
Non-viability of the gamete in species hybrids 260
Non-viability of the zygote in species hybrids 260
Incompatibility between species in Nicotiana 262
CONCLUSION 263
SUMMARY 264
LITERATURE CITED 267

INTRODUCTION

The genus Nicotiana has been a favorite subject for study since the time of KÖLREUTER, his investigation of crosses between *N. paniculata* and *N. rustica* being the first genetical research of which we have a record. Unfortunately, the work of the hybridizers of the eighteenth and nineteenth centuries produced few generalizations of value to genetics, because of the numerous variables under consideration. The foundations of genetics have been laid by experiments where a limited number of genetic differences have been followed and where the fertility of the hybrids was virtually perfect. More recently, however, there has been a trend back to the study of species hybrids, using both the cytological and the pedigree culture methods of technique. The work of SAX on the genus Triticum is an excellent example. Using the general philosophy of genetics built up by intra-specific studies as a basis of his inductions, SAX has been able to throw considerable light , not only upon the genetic behavior of the species composing this genus, but also upon their origin. It was

[1] Fellow of The International Education Board.

thought, therefore, that perhaps a similar series of investigations upon the genus Nicotiana might yield results of some value. There was the further hope that the genus might again be brought to the favorable attention of geneticists. It deserves a high place in their regard, for the plants are easy to grow, crosses are easy to make, large quantities of seed are produced, and the seed retains its viability for several years.

Since the genus is fairly large (about 60 species having been described) it has been impossible for me to make even a relatively complete survey. I have been able to obtain 19 species. In each of these forms, the chromosome number has been determined. In addition, 15 species were selected for hybridization work, and an effort made to cross each of these species with all of the others. In the case of nine hybrids, the cytological behavior of the F_1 plants was investigated.

The species used correspond with the descriptions found in COMES' Monographie du genre Nicotiana (1899), with one or two exceptions. These exceptions are species described since the appearance of this work. Their descriptions can be found in the original publications cited in the bibliography.

METHOD AND TECHNIQUE

The haploid chromosome number in each species was determined both from aceto-carmine preparation made according to Bellings' method and also from permanent slides. A very small part of the bud was cut away at the tip so that a part of each anther could be examined by the aceto-carmine method. The rest was immediately fixed with modified Bouin's solution as developed by ALLEN. Sections were cut 7 to 10 microns thick, and were stained with Haidenhain's iron-hæmatoxylin.

The diploid chromosome numbers of the pure species, and in a few cases of the dwarf species hybrids, were determined by a study of the root tips at a time when the plant, grown in sterile soil, had produced its 6th or 7th leaf. The fixative in this case was strong Flemming's solution, the stain being again Haidenhain's iron-hæmatoxylin.

In the hybridization work, 3 or 4 plants of both species were always used. The parent plants were kept together, but isolated as far as possible from the plants of the other species. The hybridization was made in the greenhouse, and in order to prevent contamination, all buds which could not be used for hybridization were cut off at a very early stage. The flowers were not bagged. Flying insects were excluded from the house by cheesecloth covers; and experiments have shown that with plants having heavy pollen, as is the case with all Nicotiana species, this is a sufficient

precaution. Crosses and reciprocals were made in each case using five buds of every combination. The seeds were always grown in sterilized soil.

THE CHROMOSOMES OF THE VARIOUS NICOTIANA SPECIES

Tabacum section

1. *N. Tabacum* L. Within this species I have made cytological studies of the following varieties: Cuba, *macrophylla*, and a white flowering tobacco. According to COMES, Cuba belongs to the var. *havanensis*. It has lanceolate leaves and pink flowers. *Macrophylla* is a very strong plant with large ovate leaves gradually tapering to a broad clasping base, with rounded but not prominent basal lobes. The flowers are deep red with a stout tube and an abruptly swollen, broad infundibulum; the limb is almost pentagonal, and at the base of every lobe is a whitish, triangular spot. The white tobacco is a small variety bearing a large number of small lanceolate and broadly decurrent leaves tapering towards the base on a relatively short stem. The corolla is relatively small, short, and cream colored.

In each of these three varieties, I found 24 chromosomes in the pollen mother cells during the different stages of the reduction division. The number was very easy to count at the second metaphase or at the first anaphase because the chromosomes at these stages, though small, are clearly separated. Sometimes the first metaphase (figure 19) is exceptionally clear and favorable for the study of chromosome size. At this stage the chromosomes are twice as large as in the second metaphase and lie close together. Figure 19 shows clearly the differences in the chromosome size. The small chromosomes are arranged in the center, the larger chromosomes at the periphery.

There is great difference in the chromosome shape at meiosis and at mitosis. In the cells of the root tips the chromosomes are long and slender, usually curled, and difficult to count. In figure 1 is represented one of the root tip cells of *N. Tabacum* var. *macrophylla* in which it is relatively easy to distinguish 48 chromosomes.

Rustica section

2. *N. rustica* L. In this species I have studied the varieties *humilis*, *brasilia*, and one which is apparently *texana*. The 24 haploid chromosomes of the pollen mother cells, in contrast to those of *Tabacum*, are uniform in size and shape. Their shape is markedly different from that of the *Ta-*

bacum chromosomes, being bean-shaped instead of spherical (figure 18). During the second metaphase they are to be seen clearly because of their separation, but are so small that their shape is vague, and they appear to be spherical.

The somatic chromosomes, though somewhat larger than those of *Tabacum*, are entwined and difficult to distinguish. It was impossible to make counts on preparations of root tips from the small *humilis* variety because the cells are very small and the chromosmes much entwined. Only in *brasilia* was I able to find clear plates (figure 2) where the chromosomes are manifestly 48.

3. *N. paniculata* L. The material used had all of the characteristics of true *N. paniculata*—a long, slender corolla tube with short, symmetrical, greenish corolla lobes. The upper leaves were lanceolate, the basal leaves were ovate, slightly cordate, and with long petioles.

The 12 chromosomes counted during different stages of the reduction division in the pollen mother cells (figure 25) seem to be identical in shape with those of *N. rustica*. In the cells of the root tips the chromosmes are very slender, long, and twisted; but 24 are easily distinguished (figure 8).

4. *N. glutinosa* L. The material used in this study was a small branched plant with small, cordate, long petioled, extremely glandular leaves. The flowers were short and cylindrical below, but suddenly swollen above into an irregular, obliquely one-sided funnel. The limb was bilabiate, the stigma and anthers being connivent just under the middle lobe of the upper lip. The color was light yellow tinged with red.

Twelve chromosomes were counted in the different stages of the pollen mother cells. The chromosomes are uniform in size and shape, the spherical shape giving them a greater resemblance to those of *N. Tabacum* than to the bean-shaped chromosomes of *paniculata* or *rustica*. They are only one-half as large as those of *paniculata*. During the first metaphase they lie very close together (figure 26), but are very clear and distinct in the first anaphase. The differences between the chromosomes of *glutinosa* and *paniculata* are visible also in the somatic cells, those of the former (figure 6) being shorter and slenderer than the latter.

5. *N. glauca* Grah. This species is a tree tobacco with long, petioled, glaucous leaves. The flowers are pale yellow with tubes similar to those of *paniculata*. The stem and leaves are bluish green. The chromosome number was found to be 12 in the pollen mother cells (figure 23) and 24 in the root tips (figure 9). The somatic chromosomes are extremely large, long, and entwined, but because of the large cells and nuclei they are well scattered and therefore are easily distinguished.

6. *N. tomentosa* R. et. P. The two year old plants are not yet in flower, but their general character shows them to be true *N. tomentosa* (*Lehmannia tomentosa* Spr.). The chromosome number was counted in the cells of the root tips and found to be 24 (figure 10).

Petunioides section

7. *N. Bigelovii* Wats. According to SETCHELL (1912) different varieties of *N. Bigelovii* exist. The plants used were small, their first leaves being slightly decurrent, nearly petioled, in fact, the later leaves becoming characteristically truncate, auriculate, and partly clasping at the base. The first leaves were more or less elongate deltoid, the later leaves more lanceolate. The corolla tube was narrow, and the corolla limb deeply divided into long pointed lobes. The corolla was pure white. Chromosome counts were made most easily during the first and second metaphases. During the first metaphase (figure 16) the chromosomes appear as bean-shaped objects about equal in size but not quite so large as those of *N. rustica* and *N. Tabacum*. In the root tips 48 chromosomes were counted (figure 3).

8. *N. viscosa* Lehm. (?). This species was raised from seeds obtained from a Gray Herbarium sheet labeled *N. viscosa*. At the same time I obtained seeds from another Gray Herbarium sheet labeled *N. attenuata*. Both groups of plants had the same morphological characters. Both lots may be *N. attenuata*, but I have labeled them *N. viscosa* (?) because I raised other specimens received from Dr. T. H. GOODSPEED as *N. attenuata* which differed somewhat from the first two lots. The latter group of plants was rather similar to *N. acuminata*, but very much smaller. These specimens, in fact, seemed to be much reduced acuminatas, and therefore correspond more closely to the taxonomic description of *N. atenuata*. I am not certain of the identification, however, for these species are quite similar to each other, and perhaps should be grouped together.

The pollen mother cells are nearly as large as those in *macrophylla*. The chromosomes also are quite large, lying close together during the first metaphase (figure 20) and more distant during the second. During the first and second metaphases as during the first anaphase I counted 24 chromosomes.

9. *N. nudicaulis* Wats. *N. nudicaulis* is a very small plant with dark green ovate leaves narrowly decurrent. The corolla is shorter and broader than in *N. trigonophylla*. The tube is somewhat brownish, the limb cream. The corolla lobes overlap.

The chromosomes of the pollen mother cells are not easy to count at any stage of the reduction division, being small and usually rather dumb-

bell shaped. In the very early first metaphase the chromosomes are somewhat more spherical, however, and 24 could be distinguished (figure 21). In the root tips, it is relatively easy to count 48 chromosomes, the chromosomes being slender and well separated (figure 4).

10. *N. suaveolens* Lehm. The plants used were branched, with the lower leaves ovate, sessile, clasping at the base and pointed at the tip. The upper leaves were sharply lanceolate and clasping. The corollas were ivory white, salver shaped and with long slender, non-infundibular tubes. The corolla limb is large with broad, shallow rounded lobes.

During the first metaphase of the pollen mother cells, the 16 chromosomes always lie close together (figure 22). They are most easily counted in the first anaphase or in the second metaphase. Cells were found with both plates of a first anaphase lying in the same section in such a manner that by focussing 16 chromosomes can be counted in each. In the first anaphase, the chromosomes are seen as curved rods or as spheres depending on their position. In both the first and the second metaphases they are more or less spherical. During the second metaphase they are more separated and are easy to count. The chromosomes in this species are relatively very small though the cells in comparison with those of the other species are very large. In the cells of the root tips 32 chromosomes could be counted. The chromosomes here are rather slender, comparatively short, and often U-shaped (figure 5).

11. *N. sylvestris* Speg. *et* Comes. In general habit as in leaf shape this species of the Petunioides section is very similar to certain *Tabacum* varieties. Its corolla is white, and very long and slender, the tube being inflated in the middle. The corolla limb consists of 5 broadly triangular lobes. The flowers ordinarily droop.

In the pollen mother cells during the heterotypic metaphase 12 spherical chromosomes, closely arranged at the plate, were counted (figure 24). The first anaphase and the second metaphase are the best for making counts, the 12 chromosomes then lying fairly distant from each other. In the cells of the root tip preparations the chromosomes are long, U-shaped, quite large, and spread out, so that it is easy to distinguish 24 chromosomes (figure 7).

12. *N. acuminata* Grah. This is a branched plant with petioled leaves, cordate below and lanceolate above. The coarse, spine-like glandular hairs on the surface and on the margin are characteristic. The corolla is white with a cylindrical tube and shallow, rounded lobes on the limb.

Twelve chromosomes were counted during the different stages of the reduction division of the pollen mother cells (figure 27). It is easier to

count them at the second metaphase or the first anaphase. The chromosomes after the second division are long and U-shaped, somewhat similar to those of the somatic cells, though during the other stages they are almost spherical. In some other plants this is a common phenomenon; but in the rest of the Nicotiana species it apparently does not occur, the chromosomes after the second division ordinarily resembling those of the earlier stages.

13. *N. trigonophylla* Dun. This is a very small plant with broadly lanceolate leaves, narrow at the base and then expanded into broad, partly clasping auricles. The corolla is very small with a short, cylindrical tube. The corolla limb consists of five broad, obtuse, and shallow lobes. The flowers are a deep cream tinged with green.

In all stages of the reduction division in the pollen mother cells 12 chromosomes were found (figure 28). The chromosomes were all of about the same size, spherical, and distinctly spread, especially during the second metaphase. In the somatic cells of the root tip they are easily distinguished as 24 relatively straight and slender chromosomes (figure 11).

Seeds from a specimen at the Gray Herbarium were obtained under the name of *N. Palmeri* A. Gray. Plants raised from them exhibited no particular differences from *N. trigonophylla*, except that the corolla was lighter and the leaves more lanceolate than those of my *trigonophylla* plants. Twelve chromosomes were counted in the different stages of the pollen mother cells (figure 29). Permanent slides of the young root tips show 24 nicely spaced chromosomes (figure 12).

14. *N. longiflora* Cav. This is quite a tall plant with broad, lanceolate or oblanceolate leaves, coarsely bullate and rugose above, the epidermis smooth except for coarse, spine-like, glandular hairs on the surface and margins. It early develops a characteristically compact rosette of large, coarse leaves which lie flat on the ground, the rosette persisting for a considerable time before the flowering stalk develops. The corolla tube is extremely long and slender. The broad, spreading limb is deeply divided into five moderately broad, blunt-pointed lobes. During the first metaphase (figure 30) and the first anaphase I counted 10 chromosomes.

15. From *N. plumbaginifolia* Viv., a species closely related to *N. longiflora*, I have obtained good preparations of the root tips in which 20 well separated chromosomes of different shapes and sizes could be clearly distinguished (figure 13).

16. *N. alata* Lk. et Otto. The plants which I have investigated came from commercial seed, and showed great variability. Concerning their origin I know nothing. They may have been segregates from

crosses between *N. alata* and *N. Forgetiana*, since it is supposed that the different colored varieties of *N. Sanderae* have originated by crossing *N. alata* with *N. Forgetiana* (HEMSLEY 1905). Some of these plants, however, seem to be the pure *alata* type. I have investigated the pollen mother cells of about 25 plants differing in habit, shape and corolla color, making both acetocarmine preparations and permanent slides. In some plants I found 8 chromosomes in all of the stages. In other plants I obtained irregular results. It was doubtful whether the number was 8 or 9, because one of the chromosomal masses could be considered as two small chromosomes lying close together, or as end portions of a single chromosome. Since I found the somatic chromosomes to be very different in shape and size, I am inclined to accept the second hypothesis. In the cells of the root tips I could distinguish 16 chromosomes; one pair very long and S-shaped, one pair long and straight, 4 pairs U-shaped and of different sizes, one pair very short and rod-shaped, and a small pair almost spherical (figure 14). Since the difference in size is so extreme in the somatic cells, the relative size proportions ought not to disappear in the pollen mother cells even though the contraction of the chromosomes there is very great. Some spindles of the first metaphase show clearly that the chromosomes are mostly long and dumb-bell-shaped, though in polar view they appear almost spherical. I am inclined to believe, therefore, that the position of some of the 4 U-shaped chromosomes may be such as to present only the tips which could appear as separate chromosomes lying close together.

It may be possible, also, that this occurrence is due to an earlier split of some of the chromosomes as described by WINGE (1917) for *Chelidonium majus* and *C. majus* var. *laciniatum*. WINGE explained BÖNICKE's determination of the chromosome number in *Chelidonium majus* as eight instead of six by the observation that the chromosomes in the pollen mother cells of this species have a marked power of precocious division. He found that "in the early anaphase, the six chromosomes moving towards the pole are not only divided into two, but into four, thus resembling a *Sarcina*, and, indeed, it would seem as if still finer subdivisions were present, which renders it difficult to ascertain the true number of chromosomes." The splitting phenomenon he found to be still more frequent in var. *laciniatun* than in the typical *Chelidonium majus*, and concluded: "The fact is of considerable interest from a theoretical point of view, as the distinct division in the heterotypic anaphase shows that the chromosome must not be regarded as an indivisible unit, but as a complexity, in which an inner differentiation or duality is already present. And in all probability,

it is also actually existent even when no outward and visible sign of division is discernible."

From the many plants in which I have studied chromosome number in the pollen mother cells I shall present some figures drawn from temporary slides made by the acetocarmine method. By this method I was able to distinguish the rod shape of the chromosomes not only in side view on the spindles, but also in polar view, for the acetocarmine fluid included between the slide and the cover glass allowed the chromosomes to move slightly and to take different positions during the observation. In cases in which questionable numbers were found, the final conclusion was drawn only after exact observations of the questionable chromosomes in different positions. In figure 34, one plate of the second metaphase was in polar view, and appeared to have 9 chromosomes; the other was not exactly in polar view, and only 7 chromosomes could be counted with certainty. After standing, however, the position of the material was changed, the plate with the 7 chromosomes appearing as a regular spindle in which the chromosomes were more or less indistinguishable, and the other plate showing clearly 8 chromosomes.

In all of the other cells counted the number of the chromosomes was always 8 (as in figure 35). Figure 36 shows the shape of the chromosomes of the first metaphase in side view. In figure 32 all chromosomes clearly show splitting, and are similar in shape to those in figure 21*b*, *d*, and *h* presented by WINGE for *Chelidonium majus*. Figure 33 is very similar to figure 21*i* of WINGE. The chromosomes can be counted as 10, but since in two places chromosomes lie so close together, I am inclined to believe that they are two long chromosomes instead of four.

GOODSPEED in a preliminary note (1923) has reported for different Nicotiana species the haploid numbers 9, 12, and 24, but for some of them he makes the following reservation.

> Three of these counts are open to some question. In the case of *N. alata*, homotypic anaphase plates in the fixed material show 10 chromosomes in some cases, although the predominating number in such stages is 9. Similarly, *N. longiflora* can be counted as 9 or 10, but in this case the predominating number is 10. Only a small amount of fixed material of *N. suaveolens* was available, and there is some doubt as to whether the number is not larger than 12, possibly 18.

Later, (1924) he describes a very interesting case of *N. alata* in which, after the second division, two of the anaphase plates have 8, and the other plates 10 chromosomes. Failure of conjugation in one chromosome pair, he believes, will account for this condition, because in other cases he found

9 chromosomes in three nuclei of the tetrads. For *N. suaveolens*, he decides that the number is 18. At the IVth International Congress of Botany at Ithaca, I showed Dr. GOODSPEED on one of my slides a single section in which both the plates of the homeotypic anaphase contained only 16 chromosomes. In a recent publication (GOODSPEED and CLAUSEN 1927), this number is admitted to be the correct one.[1]

17. *N. Langsdorffii* Weinm. This is a small plant with sessile, elliptical-lanceolate leaves, narrowed and decurrent at the base. The corolla is funnel-shaped below with a gibbous ring above and a concave, spreading limb slightly notched into five broad, shallow lobes, greenish yellow and pendent.

At the various stages, 8 chromosomes were observed (figure 31), though it is not always easy to count them for reasons similar to those discussed in the case of *N. alata*. In the root tips (figure 15), I found 16 chromosomes similar in shape to the chromosomes found in the root tips of *alata* (figure 14).

Polydiclia section

18. *N. quadrivalvis* Pursh. This is a small plant somewhat similar to *Bigelovii*, except that the leaves are not elongate deltoid but broadly deltoid. The corolla is shaped somewhat similarly to that of *Bigelovii*, but is much larger. The capsule is spherical and twice as large as that of *Bigelovii*. During the various stages of the reduction division in the pollen mother cells, 24 chromosomes were easily counted (figure 17).

[1] Since this paper was finished, Miss RUTTLE has published an interesting study of the chromosomes in *N. alata* var. *grandiflora* (Univ. of California Pub. Bot. **11**: 159–176, 1927). She has gone about her investigation in a very workmanlike manner, making extensive counts and even endeavoring to identify the individual chromosomes in various plates. She concludes that there are 18 chromosomes in the somatic cells, the usual distribution being 9–9, though 8–10 distributions occur in about nine percent of the cases. She believes that in my previous work (Jahrb. d. Univ. Sofia, Agric. Fac. **3**: 37–86, 1925) where a diploid number 16 was reported for the species, I incorrectly figured two chromosomes as one—a very long one. It is possible that Miss RUTTLE's view is correct. But I must call attention to two points which should be weighed carefully before a decision is made. First, Miss RUTTLE figures 3 pairs of U-shaped chromosomes in her Figure 1a plus 2 pairs the ends of which lie very close together (upper center). This latter configuration I believe to be a single pair of chromosomes. Second, and this is my critical point, in certain hybrids with species having 10 chromosomes as the haploid number, the reduction division of the pollen mother cells shows the number of univalents properly to be expected if the chromosome number for *N. alata* is $x=8$. For example, in heterotypic spindles of *N. longiflora*×*Sanderae*, *N. longiflora*×*alata*, *N. plumbaginifolia*×*alata*, *N. plumbaginifolia*×*Langsdorffii*, *up to two lagging univalent chromosomes* occur and are often to be seen approaching the poles, whereas the bivalents are still at the equator (see figure 37); and *regularly more than two lagging chromosomes* appear during the homeotypic division.

19. *N. multivalvis* Lindl. This is a plant very similar to *quadrivalvis*, only larger, with very large corolla and capsules. Counts were made only from temporary slides with actetocarmine staining and 24 chromosomes were counted in the pollen mother cells.

HYBRIDIZATION RESULTS

In the hybridization experiments the following species of Nicotiana were used:

1. *N. alata*
2. *N. Sanderae*
3. *N. longiflora*
4. *N. plumbaginifolia*
5. *N. acuminata*
6. *N. trigonophylla*
7. *N. sylvestris*
8. *N. suaveolens*
9. *N. Bigelovii*
10. *N. nudicaulis*
11. *N. Langsdorffii*
12. *N. paniculata*
13. *N. glutinosa*
14. *N. rustica*
15. *N. Tabacum macrophylla*

The technique of hybridization has already been described. Crosses were made between nearly all of these species in both directions, pollinating five (or more) flowers in each case. The few exceptions were the omission of *N. Sanderae* as female, of nine combinations with *N. nudicaulis*, and of one combination with *N. rustica*. The results obtained are recorded in table 1.

Two weeks after the capsules were harvested the seeds were sown. For every cross several sowings were made according to the quantity of seed harvested. If all of the flowers which were pollinated produced capsules, 100 seeds were first taken for the germination test, the rest being sown in sterilized soil for the production of F_1 plants. For crosses from which one or two capsules only were harvested, or in which only a few seeds developed, the material was used in the germination experiment. If germination occurred, the seedlings were transferred to pots of sterilized soil. If no germination occurred after three weeks, successive sowings were made until all the seed was used. The germination experiment was made in the usual way on blotting paper, the papers being kept in an incubator at a temperature of 27° to 30° C. Sometimes it happened that not a single seed germinated in the soil while a few germinated in the germination test; but it also happened that a few germinated in the soil though none appeared in the germination test.

A prospectus of all the crosses is given in table 1. In the horizontal rows are given the species used as female, and in the vertical row those used as male. The chromosome number for each of the species follows the name. The crosses in which the flowers have dropped after pollination are de-

noted by *o*, those in which the harvested capsules contained no seeds or non-germinative seeds by *d*, those which produced defective seedlings by *s*, those in which mature hybrids were produced by *h*.

TABLE 1.

Results of the hybridization experiments.

SPECIES USED AS MALES		SPECIES USED AS FEMALES														
		N. alata	*N. Sanderae*	*N. Langsdorffii*	*N. longiflora*	*N. plumbaginifolia*	*N. acuminata*	*N. sylvestris*	*N. trigonophylla*	*N. paniculata*	*N. glutinosa*	*N. suaveolens*	*N. Bigelovii*	*N. nudicaulis*	*N. rustica*	*N. Tabacum*
		X=8	8	8	10	10	12	12	12	12	12	16	24	24	24	24
N. alata	X=8	..	..	h	h	h	d	d	d	s	o	s	d	d	..	h
N. Sanderae	8	h	..	h	o	o	o	d	d	h	d	o	d	..	h	h
N. Langsdorffii	8	h	..	..	o	h	o	o	s	h	s	o	s	d	h	s
N. longiflora	10	o	..	o	..	h	d	o	d	s	d	h	s	..	d	s
N. plumbaginifolia	10	o	..	o	h	..	d	o	d	s	d	h	s	..	d	s
N. acuminata	12	o	..	o	o	o	..	o	o	o	d	d	d	..	d	o
N. sylvestris	12	o	..	o	o	o	o	..	o	d	h	o	s	o	o	h
N trigonophylla	12	o	.	o	o	o	o	o	..	d	o	o	o	h	d	o
N. paniculata	12	o	..	o	o	s	o	o	d	..	d	o	s	o	h	o
N glutinosa	12	o	..	o	o	o	o	o	d	h	..	h	h	..	d	h
N. suaveolens	16	o	..	o	o	o	o	o	d	s	d	..	h	d	s	d
N. Bigelovii	24	o	..	o	o	o	o	o	d	d	h	h	..	..	d	d
N. nudicaulis	24	o	..	o	..	..	o	..	d	d	h	o	h	..	d	d
N. rustica	24	o	..	o	o	o	o	o	d	d	o	o	o	d	..	o
N. Tabacum	24	o	..	o	o	o	d	h	d	d	d	s	h	s	h	..

Combinations marked .. were not made
Crosses marked o produced no capsules
Crosses marked d produced capsules without viable seeds
Crosses marked s produced defective seedlings
Crosses marked h produced mature hybrids

Thus *N. alata*, used as female, gave hybrids with *Langsdorffii* and *Sanderae*; but when pollinated by other species it dropped its flowers. When used as male, however, *alata* produced hybrids not only with *Langsdorffii*, but also with *longiflora*, *plumbaginifolia*, and *Tabacum*. *Langsdorffii* as female shows the same results as *alata*, but as male exhibits some differences.

Trigonophylla, which is placed by DUNAL (1852) in the *Rustica* and by COMES in the Petunioides section, produced defective seedlings when pollinated by *Langsdorffii* of the Rustica section, and with *alata* and *Sanderae* of the Petunioides section it did not even give viable seeds. *Glutinosa*

which is in the Rustica section, gave the same results when pollinated with these species. In general, the species with 12 haploid chromosomes show a very weak power of hybridization, *acuminata* and *trigonophylla* producing no hybrids with any of the species used in these experiments.

In species with 24 chromosomes the hybridizing ability is somewhat greater than in those with lower chromosome numbers, but compatibility between species shows no significant relation to taxonomic status.

Out of 186 crosses 84 were made reciprocally, and 67 of these crosses were between species differing in their chromosome number. In 56 of the latter no mature hybrids were obtained when the species with the lower chromosome number was used as female, but a higher degree of compatibility was shown when the species with the higher chromosome number was used as female. In 8 crosses where the species with the lower chromosome number was used as female the pollinated flowers dropped, while from the reciprocals mature hybrids were raised. In 4 other crosses where the species with the lower chromosome number was used as female, defective seeds were produced; while in the reciprocals mature hybrids were obtained. In 13 crosses the pollinated flowers dropped from the species with the lower chromosome number, while the reciprocals produced defective seedlings. In 19 crosses the pollinated flowers of the species with the lower chromosome number also dropped, although the reciprocals produced defective seeds. In one cross both types of crossing resulted in defective seedlings, and in another only defective seeds were produced. In 9 crosses the incompatibility between the species was so great that the pollinated flowers dropped in both the direct and reciprocal crosses. In one cross defective seeds were produced by using the species with the smaller number of chromosomes as female, and defective seedlings by the reciprocal.

In 8 reciprocal crosses the degree of compatibility appeared to be greater when the species with the lower chromosome number was used as female, but none of these crosses produced mature hybrids. In 6 of these crosses the flowers of the species with the lower chromosome number produced defective seeds, while the flowers of the reciprocals dropped after pollination. In 2 crosses defective seedlings were produced when the species with the lower chromosome number was used as female; in one of the reciprocals defective seeds were produced, in the other the flowers dropped.

Mature hybrids were obtained in both directions from only three of the 67 crosses involving species differing in their chromosome number, but in two of them (*glutinosa*×*Bigelovii* and *sylvestris*×*Tabacum*) a much

larger number of plants per capsule were obtained when the female parent had the higher chromosome number.

Mature hybrids were obtained in both directions from only two (*alata*×*Langsdorffii* and *longiflora*×*plumbaginifolia*) of the 17 reciprocal crosses between species having the same chromosome number. Of 10 reciprocally made crosses between species with 12 haploid chromosomes, 4 did not produce seeds, no matter which parent was used as female. In 3 crosses the flowers dropped after pollination, while their reciprocals produced defective seeds. In 1 cross defective seeds were produced by both parents. From 2 crosses (*glutinosa*×*sylvestris* and *paniculata*×*glutinsoa*) a few very weak mature hybrids were raised, although their reciprocals produced respectively defective seeds and nothing.

Out of 5 reciprocally made crosses between species with 24 haploid chromosomes, 2 produced very vigorous hybrids, while their reciprocals produced defective seedlings and nothing. In a third cross defective seedlings were produced in one direction and defective seeds in the other. In the fourth cross both female parents produced defective seeds. In the last cross the seeds were defective in one female parent and the flowers dropped in the other.

DESCRIPTION OF THE SPECIES HYBRIDS

A. Species hybrids which did not reach maturity.

Of the various crosses made, 19 produced hybrids which died in early seedling stages.

a. Hybrids obtained by crossing species having 12 with species having 8 as the haploid chromosome numbers are:

1. *N. trigonophylla*×*Langsdorffii*
2. *N. glutinosa*×*Langsdorffii*
3. *N. paniculata*×*alata*.

b. Hybrids obtained by crossing species having 12 and 10 as the haploid chromosome numbers are:

4. *N. paniculata*×*longiflora*
5. *N. paniculata*×*plumbaginifolia*
6. *N. plumbaginifolia*×*paniculata*.

c. Hybrids obtained by crossing species having 16 and 8 as the haploid chromosome numbers are:

7. *N. suaveolens*×*alata*.

d. Hybrids obtained by crossing species having 12 and 16 as the haploid chromosome numbers are:

8. *N. paniculata*×*suaveolens*.

e. Hybrids obtained by crossing species having 24 and 8 as the haploid chromosome numbers are:
   9. *N. Bigelovii* × *Langsdorffii*
   10. *N. Tabacum* var. *macrophylla* × *Langsdorffii*.
f. Hybrids obtained by crossing species having 24 and 10 as the haploid chromosome numbers are:
   11. *N. Bigelovii* × *longiflora*
   12. *N. Bigelovii* × *plumbaginifolia*
   13. *N. Tabacum* var. *macrophylla* × *longiflora*
   14. *N. Tabacum* var. *macrophylla* × *plumbaginifolia*.
g. Hybrids obtained by crossing species having 24 and 12 as the haploid chromosome numbers are:
   15. *N. Bigelovii* × *sylvestris*
   16. *N. Bigelovii* × *paniculata*.
h. Hybrids obtained by crossing species having 24 and 16 as the haploid chromosome numbers are:
   17. *N. rustica* × *suaveolens*
   18. *N. suaveolens* × *Tabacum* var. *macrophylla*.
i. Hybrids obtained by crossing species having 24 and 24 as the haploid chromosome numbers are:
   19. *N. nudicaulis* × *Tabacum* var. *macrophylla*.

Of these crosses, only *N. suaveolens* × *Tabacum* var. *macrophylla* (germination 82 percent), *N. Tabacum* var. *macrophylla* × *plumbaginifolia* (germination 3 percent), and *N. nudicaulis* × *Tabacum* var. *macrophylla* (germination 16 percent) yielded a great number of seedlings. In all other crosses the germination was very low. In the cross *N. Tabacum* var. *macrophylla* × *plumbaginifolia*, the seedlings all died after developing the fourth or fifth true leaf. In most of the crosses the seedlings died before developing the second true leaf.

B. Species hybrids which reached maturity.
   a. Hybrids obtained by crossing species having 8 as the haploid chromosome number.

I. *N. alata* × *Sanderae* (Cl_{10})

The germination of the seeds obtained was 81 percent. The ten hybrids raised were sterile. They were vigorous but variable, which may indicate that *Sanderae* is heterozygous for various genes, since in other species crosses where *Sanderae* was used the hybrids were also variable.

Nearly all of the hybrids resembled *N. Sanderae* in habit of growth. The leaves were an intermixture of the characteristics of both parents. In corolla color the *Sanderae* parent was dominant; but in size and shape the flowers were nearly intermediate.

II. *N. alata* × *Langsdorffii*

The germination of the seeds obtained was 80 percent (93 percent in the reciprocal). The ten hybrids raised were fertile, vigorous, uniform, and in habit of growth resembled *Langsdorffii*. The leaves were nearly intermediate in color, size and shape. The flowers also were intermediate in color, size and shape. The pollen-grains were intermediate in color—slightly bluish.

III. *N. Langsdorffii* × *Sanderae* (Cl_{10})

The germination of the seeds obtained was 62 percent. The ten hybrids raised were fertile, vigorous, and variable in habit of growth, though resembling *Langsdorffii*. The leaves were different in size and shape in the different individuals. In respect to corolla color, *Sanderae* was dominant. In size and shape the flowers were different in the different individuals. The pollen-grains were intermediate in color—light bluish.

b. Hybrids obtained by crossing species having 10 and 8 as the haploid chromosome numbers.

IV. *N. longiflora* × *alata*

The germination of the seeds obtained was 95 percent. The fifty hybrids raised were sterile. They developed slowly into vigorous, uniform plants, in habit of growth an intermixture of the characteristics of both parents. The leaves were slightly rugose, like those of *longiflora*. The flowers, which remained slightly closed during the day, were intermediate in size and shape, though with the corolla lobes bluish externally as in *longiflora*.

V. *N. longiflora* × *Sanderae*

I was unsuccessful in this cross; but ten hybrids were raised from seeds obtained by DR. A. J. MANGELSDORF. The plants were sterile; and though variable in size and in habit of growth, were quite vigorous. The leaves of each individual were slightly rugose like those of *longiflora*. The flowers, although somewhat variable, resembled the two parents in size and shape. They remained slightly closed during the day like those of *longiflora*.

VI. *N. plumbaginifolia* × *alata*

The germination of the seeds obtained was 49 percent. The fifty hybrids raised were sterile. They developed slowly to vigorous, uniform plants, in habit of growth an intermixture of the traits of both parents. The leaves were slightly rugose like those of *plumbaginifolia*. The flowers were intermediate in size, bluish externally like *plumbaginifolia*, and remained slightly closed during the day.

VII. *N. plumbaginifolia* × *Langsdorffii*

The germination of the seeds obtained was 35 percent. The thirty hybrids raised were sterile. They were vigorous, uniform, and in habit of growth intermediate between the parents. The leaves resembled *Langsdorffii* in color, but were slightly rugose like those of *plumbaginifolia*. The flowers were intermediate in size, shape and color. The corolla lobes were yellowish on the upper side, but slightly bluish underneath. The pollen-grains were intermediate in color—slightly bluish.

c. Hybrids obtained by crossing species both having 10 as the haploid chromosome number.

VIII. *N. longiflora* × *plumbaginifolia*

The germination of the seeds obtained was 96 percent (81 percent in the reciprocal). The ten hybrids raised were fertile, very vigorous, uniform, and in habit of growth intermediate between the parents. The leaves and the flowers were also intermediate in their characteristics.

d. Hybrids obtained by crossing species having 12 and 8 as the haploid chromosome numbers.

IX. *N. paniculata* × *Sanderae* (Cl_{10})

The germination of the seeds obtained was 28 percent. The ten hybrids raised were sterile. They varied markedly in vigor, from very weak to very vigorous; they also varied in habit of growth. The leaves were variable in the different individuals, but were nearly all petiolate as in *paniculata*. The flowers in all were as red as those of the *Sanderae* parent, varying in size but more closely resembling *paniculata* in shape.

X. *N. paniculata* × *Langsdorffii*

The germination of the seeds obtained was 12 percent. The ten hybrids raised were sterile. The plants were uniformly very weak, in habit of growth resembling *Langsdorffii*. The petiolate leaves resembled *paniculata*

rather closely. The flowers in size and shape resembled *Langsdorffii*. The pollen-grains were intermediate in color—slightly bluish.

e. Hybrids obtained by crossing species both having 12 as the haploid chromosome number.

XI. *N. paniculata* × *glutinosa*

The germination of the seeds obtained was less than 1 percent. The five hybrids raised were sterile. The plants were very weak at the beginning, but quite vigorous at the end. They exhibited little variablity, and were an intermixture of the characteristics of both parents. The first leaves were oblong, the later ones cordate, thus resembling *glutinosa*. The flowers were yellow, tinged with pink, and were intermediate in size and shape.

XII. *N. glutinosa* × *sylvestris*

The germination of the seeds obtained was 5 percent. The two hybrids raised were sterile. They developed slowly and were very weak, though uniform and in habit of growth intermediate between the parents. The leaves were nearly petiolate as those of *glutinosa*, but oblong like those of *sylvestris*. The flowers were pink as in *glutinosa*, but in size and shape intermediate.

f. Hybrids obtained by crossing species having 16 and 10 as the haploid chromosome numbers.

XIII. *N. suaveolens* × *longiflora*

The germination of the seeds obtained was 22 percent. The thirty hybrids raised, though sterile, were very vigorous and in habit of growth resembled *suaveolens*. The leaves also resembled *suaveolens*. The flowers were more like *suaveolens* in size and shape; but in corolla color *longiflora* was dominant, the corolla lobes being bluish underneath.

XIV. *N. suaveolens* × *plumbaginifolia*

The germination of the seeds obtained was 2 percent. The fifteen hybrids raised, though sterile, were very vigorous, uniform, and in habit of growth resembled *suaveolens*. The leaves and flowers resembled *suaveolens*, though in corolla color *plumbaginifolia* was dominant, the corolla lobes being bluish underneath.

g. Hybrids obtained by crossing species having 16 and 12 as the haploid chromosome numbers.

XV. *N. suaveolens*×*glutinosa*

The germination of the seeds obtained was 88 percent. The five hybrids raised were sterile, very vigorous, uniform and in habit of growth resembled *suaveolens*. In size and shape the leaves were intermediate, but petiolate as those of *glutinosa*. The flowers were pink like those of *glutinosa*, but in size and shape resembled *suaveolens*.

h. Hybrids obtained by crossing species having 24 and 8 as the haploid chromosome numbers.

XVI. *N. Tabacum, Cuba*×*alata*

The germination of the seeds obtained was less than 1 percent. The two similar hybrids raised were sterile, very weak, and in habit of growth resembled *Tabacum*. The characteristics of both leaves and flowers were more nearly like those of *Tabacum*.

XVII. *N. Tabacum*, white tobacco×*Sanderae* (Cl_{10})

The germination of the seeds obtained was less than 1 percent. Three hybrids were raised from a cross in which *Sanderae* Cl_{10} was used. All of them were sterile, quite weak, and though different, were more nearly like *Tabacum*. The leaves and the flowers resembled *Tabacum* in size and shape, but the corolla color of *Sanderae* was dominant. From another cross, where *Sanderae* N16 with bluish corolla was used, the four hybrids obtained showed the result of segregation in corolla color, three being bluish and one white. These hybrids also were sterile, weak, and variable in habit, though resembling *Tabacum*.

XVIIII. *N. rustica*×*Sanderae* (Cl_{10})

The germination of the seeds obtained was less than 1 percent. The four hybrids raised were sterile. The plants varied markedly in vigor and size, although in habit of growth they resembled *rustica*. The leaves of all were petiolate, and in size and shape were like those of *rustica*. The flowers although different in size and shape in the different individuals, resembled *rustica*, except that the red corolla color of *Sanderae* was dominant.

XIX. *N. rustica*×*Langsdorffii*

The germination of the seeds obtained was less than 1 percent. The only hybrid raised was sterile, very weak and in habit of growth like *rustica*. The leaves were petiolate, resembling *rustica*, in size and shape. The

flowers also resembled *rustica* in size and shape. The pollen grains were intermediate in color—slightly bluish.

i. Hybrids obtained by crossing species having 24 and 12 as the haploid chromosome numbers.

XX. *N. nudicaulis*×*trigonophylla*

The germination of the seeds obtained was 46 percent. The twenty hybrids raised were sterile, though quite vigorous, uniform and with a habit of growth like *nudicaulis*. The leaves were also more like those of *nudicaulis*. The corolla limb was curved as in *nudicaulis*, but the tube intermediate between those of the parents in length.

XXI. *N. glutinosa*×*nudicaulis*

The germination of the seeds obtained was 10 percent. The twenty-eight hybrids raised were sterile, very weak, uniform and in habit of growth an intermixture of the characteristics of both parents. In color, the leaves were like those of *nudicaulis*, but petiolate as in *glutinosa*. The flowers were like those of *glutinosa* in color and shape, and like those of *nudicaulis* in size.

XXII. *N. Bigelovii*×*glutinosa*

The germination of the seeds obtained was 98 percent, but less than 1 percent in the reciprocal. The fifty hybrids raised were sterile, vigorous, uniform and in habit of growth resembling *Bigelovii*. The leaves were petiolate like those of *glutinosa*, but in size, shape and color resembled *Bigelovii*. The flowers were slightly pink, but in size and shape resembled *Bigelovii*.

XXIII. *N. Tabacum* var. *macrophylla*×*sylvestris*

The germination of the seeds obtained was 50 percent. The seventy-five hybrids raised were sterile, very vigorous, uniform and in habit of growth resembled *Tabacum*. The leaves and the flowers resembled *Tabacum*, but the corolla tube was more slender, the corolla limb smaller and the corolla lobes narrower than those of *Tabacum*. The reciprocal cross was made by using another *Tabacum* variety as the male parent. Here the germination of the seeds obtained was less than 1 percent. The three hybrids raised behaved in respect to the parental characters as in the cross *macrophylla*×*sylvestris*.

XXIV. *N. Tabacum* var. *macrophylla*×*glutinosa*

The germination of the seeds obtained was 25 percent. The twelve hybrids raised were sterile, very weak, variable in size and habit, an intermixture of the traits of both parents. The leaves were petiolate, small and ovate, more like those of *glutinosa*. The flowers were as red as those of *macrophylla*, but in size and shape were intermediate.

XXV. *N. rustica*×*paniculata*

The germination of the seeds obtained was 35 percent. The fifteen hybrids raised were partially fertile, quite vigorous, uniform and in habit of growth resembled *rustica*. The leaves and the flowers approached closely to those of *rustica*, but the corolla tube was somewhat intermediate in length.

j. Hybrids obtained by crossing species having 24 and 16 as the haploid chromosome numbers.

XXVI. *N. Bigelovii*×*suaveolens*

The germination of the seeds obtained was 84 percent (98 percent in the reciprocal). The thirty-three hybrids raised were sterile, very vigorous, uniform and in habit of growth resembled *Bigelovii*. The leaves and flowers were more like those found in *Bigelovii*.

k. Hybrids obtained by crossing species having 24 as the haploid chromosome number.

XXVII. *N. Bigelovii*×*nudicaulis*

The germination of the seeds obtained was 15 percent. The twenty hybrids raised were sterile, vigorous, uniform and in habit of growth an intermixture of the traits of both parents. The leaves resembled *Bigelovii*. The flowers were intermediate in color, but were small with curved corolla as in *nudicaulis*.

XXVIII. *N. Bigelovii*×*N. Tabacum* var. *macrophylla*

The germination of the seeds obtained was 15 percent. The twenty-five hybrids raised were sterile, very weak, uniform and in habit of growth an intermixture of the traits of both parents. The leaves and flowers were also intermediate.

XXIX. *N. rustica*×*N. Tabacum* white tobacco

The germination of the seeds obtained was less than 1 percent. The two hybrids raised were slightly fertile, vigorous, and in habit of growth inter-

mediate. The leaves were petiolate, and in shape were more nearly like *rustica*; but were intermediate for size. The flowers were intermediate in color, size and shape.

CYTOLOGICAL OBSERVATIONS ON CERTAIN NICOTIANA SPECIES HYBRIDS

A. *N. alata* × *Langsdorffii*

The heterotypic and homeotypic divisions in the pollen mother cells are normal.

B. *N. longiflora* × *Sanderae*

The reduction division shows a picture very similar to that of the Drosera hybrids (ROSENBERG 1909). There are eight bivalents and two univalents. Sometimes it appears that in an earlier stage of the heterotypic metaphase the univalents are in the same plane as the bivalents, but frequently one observes spindles with two lagging chromosomes (figure 37). During the second metaphase (figure 38), two univalent chromosomes regularly lag outside both equatorial plates. In figure 39, one of the univalents has already split. During the first and second divisions the univalents are usually distributed at random to the poles in a normal manner, but cases in which the 4 univalent chromosomes are lagging (figure 40) are frequently observed.

C. *N. Tabacum* × *alata*

The reduction division here is quite irregular. In the first division as many as 8 gemini can be distinguished (figure 41 and 43). Figure 41 shows the chromosomal arrangement at the multipolar spindle immediately after diakinesis. Figure 42 shows the next stage with the chromosomes aligned on the equatorial plate. Such figures are rather infrequent, however, for soon after this stage, the univalents pass to the poles. The first metaphase spindle most commonly seen is shown in figure 43. Eight bivalents and three univalents lie in the equatorial plate, the rest of the univalents being on the way to the poles. It seems that soon after some of the univalents have reached the poles, the bivalents begin to divide (figure 44). Figure 45 represents a situation occasionally occurring in which there has been precocious division of some of the chromosomes either during the final stages of the first division or at the beginning of the second division, the chromosomes of both plates plus the lagging one are $18+19+1=38$ instead of 32. Presumably 6 chromosomes have already divided. Such chromosomes, that is to say those which have gone through the equational division at the end of the first division, seem to behave during the

second anaphase much as do those of the Triticum hybrids (SAX 1922, KIHARA 1924, THOMSON 1926). I think that the fairly large number of lagging chromosomes found during the second division (figure 46) are not merely descendants of the few chromosomes which lagged during the first division, but also of those which have not undergone the equational division at the same time as the majority.

D. *N. Tabacum*×*Sanderae* (Cl_{10})

The reduction division in these hybrids is similar to that found in the hybrids of *N. Tabacum*×*alata*. Diads were frequently found.

E. *N. paniculata*×*Langsdorffii*

No bivalent chromosomes were observed during the various stages from early prophase to the second division. In figure 47, a heterotypic spindle, four of the chromosomes are very long and slender; but it is clear from the preparation that they are attached at both ends to spindle fibers. In a slightly later stage (figure 48), two such chromosomes are shown so securely fastened to a fiber that they seem to be pulled apart. At the end of the first division the chromosomes are regularly distributed between both poles, and during the second metaphase the number of lagging chromosomes is usually small (figure 49). An exceptional case is illustrated in figure 50 where all chromosomes lie in the same plane. The second division is virtually an ordinary equational division (figure 51).

F. *N. rustica*×*paniculata*

In the heterotypic metaphase of this hybrid the 12 bivalent chromosomes are easily distinguished from the 12 univalents. The bivalents are about twice as large and somewhat longer than the univalents, and are always found on the equatorial plate. The spherical univalents are scattered. Figure 52 shows the 12 bivalents in the center, 5 univalents on one side, 6 univalents on the other side, and one univalent overlapping one of the bivalents. The group of 5 lies in one plane, the group of 6 in another plane, while the equalorial plate with the 12 bivalents lies between them.

The arrangement of the chromosomes during the heterotypic anaphase is illustrated by figure 53. The bivalents, which divide soon after the univalents approach the poles, are found together with the univalents near the poles. Figures 54 and 55 show two sets in the second metaphase. In the first figure the chromosome number of the two plates is $18+18=36$, in the second $16+20=36$. This indicates that no univalent chromosomes

had split during the first division. This is the case for all counts made at this stage. (Cases of lagging chromosomes were also found). The second division is usually very regular (figure 56) though figures with lagging chromosomes have occasionally been observed.

G. *N. suaveolens*×*glutinosa*

During the first metaphase the chromosomes of both parents, 16+12=28, are scattered throughout the spindle, with no bivalents visible (figure 58). At the heterotypic anaphase the chromosomes are distributed at random to the two poles, lagging chromosomes being quite common. Figure 57 illustrates a second metaphase with 9 chromosomes in one equatorial plate and 17 in the other, while outside there are 2 lagging chromosomes. Figure 59, of the same stage, shows 4 chromosomes on a small spindle, 2 chromosomes outside, and 22 chromosomes on the equatorial plate. The chromosomal distribution during this stage is quite variable. The second division is chiefly equational. The chromosomes which lag during this division seem to be descendants of univalent chromosomes which, at the beginning of the second division, failed to reach the equatorial plates. The tetrads show a great deal of degeneration. Very often, in acetocarmine preparations, they stuck together, appeared to be shrunken, and failed to stain.

H. *N. suaveolens*×*Bigelovii*

During the heterotypic division it is very difficult to distinguish the metaphase from the anaphase, nevertheless it is easy to see that the chromosomes do not pair. The chromosomes of both parents (16+24) are scattered along the spindle. Figure 60a-b shows 37 chromosomes lying in one section and three in the other. Because the chromosome distribution in the first division is so variable the second metaphase looks quite different in different cells. Figure 61 shows 21 chromosomes lying on one plate, 16 on the other plate, and 3 lagging. Figure 62 shows the same stage in which the chromosomes are so distributed that 38 chromosomes lie on one plate, while 2 are arranged as a small spindle. The spindle fibers in this case are not visible, but the position of the chromosomes indicates a spindle. Figure 63 presents a second metaphase in which the chromosomes are distributed among three very well formed spindles, with 2 chromosomes lagging in a spindle position. The second division is regular. At this stage the chromosomes of the different spindles normally undergo the equational split simultaneously, and any lagging chromosomes which are found during and after the second division are presumably descendants

of the lagging chromosomes seen during the first division. In figure 64, showing the end of the second division, there are 8 nuclei visible. In three of the small nuclei there are 6 chromosomes; in another nucleus there are 5 chromosomes; in two other nuclei there are 10 chromosomes; while in the two very large nuclei there are 19 and 13 (?) chromosomes respectively. It is very difficult to count accurately in such a stage since the prochromosomes lie in so many different planes. I am inclined to believe that in this case, however, both of the very large nuclei really contain 19 chromosomes, the two next largest contain 10, two of the smaller ones have 6, and the other two only 5 chromosomes, originating through a chromosome distribution during the first division in which the formation of four spindles containing $19+10+6+5=40$ chromosomes took place at the beginning of the second division. The degeneration begins at the second telophase. The protoplasm is almost always very poorly fixed, the cross walls being shrunken and the nucleus itself taking the stain irregularly. It may be that the disperison of non-homogeneous chromatin between so many nuclei is due to the acceleration of the degeneration.

I. *N. rustica*×*Tabacum*, white tobacco

The first reduction division is very irregular. Although I examined many slides showing the heterotypic division there is so much variation that I could not be certain whether gemini were formed or not. In the two serial sections of figure 65 a and b, a very common situation is illustrated in which the 48 chromosomes scattered on the spindle can be easily distinguished. In some cases I have been unable to count 48 chromosomes, which I assume to be due to the presence of loose gemini.

During the second metaphase the chromosomes are distributed irregularly to both poles, some of them lagging on either side of the equatorial plates. In figure 67 is illustrated the most unequal distribution found; 36 chromosomes in one plate, 11 in the other plate, and 1 chromosome lagging between them. In anthers showing different stages of the second division, especially of the second metaphase, fairly normal single spindles were visible which were twice as large as ordinary second division spindles. Similar cases are reported by Miss LJUNGDHAL (1922) in the cross *Papaverum somniferum*×*orientale*. Such cases were thought by her to have originated through the fusion of two second metaphase spindles. Figure 66 corresponds roughly to figure 5d of her plates, though the two cases are somewhat unlike because of the different type of division. Figure 68 illustrates a case in which a cell wall has already developed between the two spindles. It is not impossible that the peculiar chromosome

distribution caused the development of the cell wall thus halting the second division just as it was starting, the result being the formation of the diads frequently found in the pollen mother cells of this hybrid. Normally the second division is very irregular, with many lagging chromosomes scattered over the two spindles. In figure 69a,b,c, the chromosomes lying in the plane of one spindle are represented in solid black, those of the other spindle in stipple, the lagging chromosomes in outline.

DISCUSSION

Inheritance in species hybrids

The gradual growth of the chromosome theory of heredity has led geneticists to interpret the anomalous distribution of characters in certain species crosses to an abnormal distribution of the chromosomes. Where the hybrids are fertile and the chromosome behavior is that which is ordinarily found in varietal crosses, the normal type of inheritance obtains (*Vide Mirabilis jalapa*×*M. longiflora*, CORRENS 1909; *Antirrhinum majus*×*A. molle*, BAUR 1911, LOTSY 1912; *Nicotiana Langsdorffii*×*N. alata*, EAST 1916). In a few cases, even partially fertile hybrids have shown a behavior similar to that found in intervarietal crosses (*Godetia Whitneyi*×*G. amœna*, RASMUSSON 1920). In general, however, one cannot go very far in a pedigree culture analysis of the results of species hybridization; though it appears probable, following STURTEVANT's work on *Drosophila melanogaster* and *D. simulans* (1920, 1921) that related species may have genes in common and may produce similar mutants.

Lack of sufficient analysis in intervarietal crosses prevents any examination of Nicotiana species hybrids after the manner used for Drosophila. SACHS-SKALINSKA (1921), however, has demonstrated Mendelian segregation for corolla color in the fertile hybrid, *N. Langsdorffii*×*Sanderae*. The colored corolla of *N. Tabacum* var. *macrophylla* which is dominant in intervarietal crosses with white tobacco has also proved dominant in the cross with *N. sylvestris*, and in somewhat diluted form in the cross with *N. Bigelovii*. The red corolla color of a *N. Sanderae* variety proved dominant over the white of *N. alata* and white tobacco. This corolla color of *N. Sanderae* was also dominant over the yellow-greenish corolla color of *N. Langsdorffii*, *N. paniculata*, and *N. rustica*. An intermediate corolla color occurred in the hybrid *N. Bigelovii*×*glutinosa* as well as in *N. Tabacum* var. *Cuba*×*alata* and *Cuba*× white tobacco. In intervarietal crosses of *Tabacum* it has been found (SETCHELL, GOODSPEED, and CLAUSEN 1922) that the petioled leaf shape is more or less dominant over

the sessile. Similarly, the petioled leaf character of *N. rustica*, *N. paniculata*, and *N. glutinosa* proved dominant in the various interspecific crosses involving these species.

The various hybrids derived by crossing species having the same chromosome number show a mixture of the parental characters due, presumably, to lack of dominance or to a similar number of dominants contributed by each parent. Where, on the other hand, the hybrids are obtained by crossing species widely differing in their chromosome number, as *N. Tabacum*×*Sanderae*, *N. Tabacum*× *sylvestris*, *N. rustica*×*Langsdorffii*, *N. suaveolens*×*longiflora*, *N. suaveolens*×*plumbaginifolia*, the hybrid resembles the parent having the larger chromosome number except in the cross *N. paniculata*×*Langsdorffii* in which the latter species appears to be dominant apart from the petioled condition of the leaves. It is not impossible that this resemblance to *N. Langsdorffii* is due to a few striking dominant characters contributed by it.

The characteristics of the sterile hybrid *N. Tabacum*×*sylvestris*, in which *N. Tabacum* is better represented, led GOODSPEED and CLAUSEN (1916) to conclude that distinct reaction systems are involved in species crosses, and that the phenomenon must be viewed in the light of a contrast between systems rather than between specific factor differences. Although they have, in some instances, recognized the influence of *N. sylvestris* on the hybrid, these authors have questioned the plate in BAUR's textbook in order to establish the complete dominance of *N. Tabacum*. KLEBS (1917), however, claims an intermediate character for the flower of this hybrid, and the intermediate corolla length of the hybrid can be distinguished in plates given by GOODSPEED and CLAUSEN (1917). Although the calcyina flower is almost completely recessive in intervarietal *Tabacum* crosses (SETSHELL, GOODSPEED, and CLAUSEN 1922), it appears in the *N. Tabacum*×*sylvestris* hybrid, albeit in not so extreme a form as in the parental types. The probability is that the twelve chromosomes of *sylvestris* do not carry the factors necessary to limit the appearance of this character, and that in general, the great resemblance to *Tabacum* is due to the transmission to the hybrid of a larger number of dominant factors with the larger number of chromosomes. The high degree of sterility, on the other hand, depends (EAST 1915, 1921) on the interrelation between the chromosomes.

A species hybrid parallel to that cited by Goodspeed and Clausen is found in crosses between white tobacco and two different *N. Sanderae* plants. The hybrids obtained show a high degree of resemblance to the *N. Tabacum* parent, but corolla color of the *Sanderae* parent is dominant.

Non-viability of the gametes in species hybrids

In the matter of sterility through the production of non-viable gametes, it is necessary to distinguish between completely sterile and partially sterile hybrids. The first category is the one most frequently found in *Nicotiana*; the second I have found only in *N. rustica*×*paniculata*. From cytological studies of different hybrids of the first category, such as *N. Bigelovii*×*suaveolens*, *N. suaveolens*×*glutinosa*, and *N. paniculata*× *Langsdorffii*, complete sterility seems to be the logical expection, since no pairing of chromosomes occurs during the first division. The chromosomes are distributed irregularly between the two poles of the heterotypic spindle, some being left behind; or, as in *N. Bigelovii*×*suaveolens*, they are distributed among three or four homeotypic spindles. In *N. rustica*×*paniculata*, on the other hand, there is distinct pairing of the chromosomes. Presumably this should afford a greater expectation of the production of viable gametes, though there are cases known where a seemingly normal reduction does not lead to viable gametes.

Of some interest is the relation between the chromosome behavior and the morphological appearance of the hybrids. *N. paniculata* and *N. Langsdorffii* both belong to the *rustica* section, but they produced very weak hybrids with no pairing of chromosomes. It seems that the mode of the reduction division is not correlated with the vigor of the hybrid, nor even with the relationship between the species, although this case is somewhat questionable as the accepted taxonomic position of *Langsdorffii* is open to criticism (Lock 1909). *N. suaveolens* and *Bigelovii*, both of the *Petunioides* section, must have some degree of genetic compatibility as demonstrated by the extreme vigor of their hybrids, but again there was no pairing of chromosomes in the reduction division, In the hybrid *N. suaveolens*×*glutinosa* I again found hybrid vigor and non-pairing of chromosomes, but here the parents are not closely related taxonomically.

It is to be noted that regularly in species hybrids of Triticum and other genera, the longitudinal split of the univalent chromosomes occurs during the first division (KIHARA 1924, SAX 1922, THOMSON 1926); but in Nicotiana in all the species hybrids studied no longitudinal split of the univalent chromosomes occurred during the first metaphase.

Non-viability of the zygote in species hybrids

Sterility in plant and animal hybrids is sometimes due to the fact that the zygote dies during its development. Such cases are found very frequently in Drosophila, where they are due to definite lethal or semi-

lethal factors (BRIDGES, MORGAN and STURTEVANT 1925). I have observed some interesting cases of zygote mortality in certain Nicotiana hybrids, such as *N. suaveolens*×*Tabacum* var. *macrophylla*. The cross is easily made and the germination of the seeds is nearly normal. After germination the seedlings start their growth at a rapid rate with a tendency to hybrid vigor until the first true leaves appear. At this stage the seedling growth stops, and after a time the plants die. This phenomenon was confirmed by three or four successive sowings. The leaves of the seedlings were a normal green as if no disturbance had occurred in the chlorophyll development. The root system, however, which was quite normal at the beginning, began to degenerate rather early although the leaves remained green a long time after. This was characteristic of all hybrids which died during vegetative development. This same cross was successful for SAGERET and for GÄRTNER, and their plants reached maturity. It is rather peculiar that in the reciprocal cross the pollinated *Tabacum* flowers dropped soon after pollination, although crosses were almost always successful when using species with the higher chromosome number as female.

The hybrid *N. Tabacum* var. *macrophylla*×*glutinosa* stopped its growth in a later stage, after from 5 to 10 leaves had developed. The root system which was very well developed degenerated with the cessation of growth, but long after that the plant was still green. I was inclined to believe that some pathological organism was responsible for the death of the roots, although the neighboring plants of other crosses were normal. I cut away the old root of several plants, disinfected them in Uspulun, and grew them in sterilized soil under artificial light 3–4 hours during the night, as the day was very short at that time. The plants recovered, and after only one week I was able to see a new root developing normally. New leaves were formed, and in three weeks the plants came into flower. Sister plants growing without artificial light never came to flower. It seems that for some reason metabolism was abnormal, and that this defect was partially nullified by increased exposure to light. This success encouraged me to try defective plants of hybrid combinations under similar conditions, and I was especially hopeful of the hybrid *N. Tabacum* var. *macrophylla*×*plumbaginifola*; but had no success. Thirty plants from two sowings were observed. In both cases the seedlings started with a very rapid growth, but stopped after the 3d or 4th leaf developed.

The single seedlings obtained from *N. Tabacum* var. *macrophylla*×*Langsdorffii* and from *N. Tabacum* var. *macrophylla*×*longiflora* had deformed cotyledons and no visible growing point. The same *N. paniculata*

plant was pollinated by three different *alata* plants and in two of the crosses the seedlings died after developing the fourth true leaf. In this case I am not sure whether they died because of their genetic constitution or because of the greenhouse conditions during the winter, or, perhaps, a combination of both. One plant of *N. Tabacum* var *Cuba* was pollinated by two different *alata* plants and by a *Sanderae* plant. With one of them, *alata*, No. 20, three capsules were obtained, but not a single seed germinated. With *alata*, No. 25, the seedlings produced died in different stages during their development and only two finally reached maturity. With *Sanderae*, No. 16, four seedlings reached maturity. The chromosomes were counted in the root-tips of some of the hybrids seedlings of *cuba*×*alata* No. 25, which died after developing the fourth true leaf and the number was found to be over 30.

From the cross *N. Tabacum* var. *macrophylla*×*nudicaulis* I obtained plump seeds having endosperms, but which did not germinate. I do not know whether the seeds contained embryos or not, as I did not make a histological investigation.

Incompatibility between species in Nicotiana

The degree of compatibility between different species is supposed to be indicated by their taxonomic relationship. This study has shown that, with some exceptions, hybridization is successful more frequently when the species with the higher chromosome number is used as female. While these results are not entirely in accord with those of other investigators, I believe that this correlation holds in Nicotiana. The literature of genetics affords no clearly parallel case. From the hybridization results between the various Triticum species as reported by different investigators, it is not clear in which direction the cross is easier, but it is evident that the species with the lower chromosome number is generally used as the female parent. Of the 26 crosses reported (TSCHERMAK 1914, SAX 1922, KIHARA 1924) in only 4 is the species with the higher chromosome number used as the female. KIHARA reports successful pollination of a species in the Emmer group (n = 14) by one of the *monococcum* group (n = 7). The other three cases are reported by SAX who successfully pollinated three species of the *vulgare* group (n = 21) by three of the Emmer group. Similar results have been reported for Papaverum species crosses, where 4 out of 17 hybrids (FOCKE 1881, LJUNGDAHL 1924) were produced by using pollen of species with the higher chromosome number. Unfortunately, these investigations do not make clear just what crosses were attempted, and which were successful.

CONCLUSION

The chromosome numbers of the different Triticum species (SAKAMURA 1918, SAX 1918) have been found to be what should be expected from the taxonomic arrangement of the species, the interspecific hybridization results, the serological studies, and resistance to parasites. The three sections of the genus are represented by three different numbers, each found only among the species of a given section, and the three numbers establish the polyploid series 2x, 4x, 6x, with the basic number 7. In Fragaria (LONGLEY 1926, ICHIJIMA 1927) the situation is similar. In Nicotiana, however, as in Chrysanthemum (TAHARA 1921), and Rosa (TÄCKHOLM 1922), each taxonomic section contains more than one characteristic chromosome number. Species with an aberrant number of chromosomes in a polyploid series, as in *longiflora* and *plumbaginiolia* in Nicotiana are frequently observed in various genera. KUWADA (1919) assumed for maize that transverse segmentation of some of the chromosomes is a genetically fixed phenomenon. HEILBORN (1924), however, believes that in Carex the higher chromosome number in various related species arose practically always through non-disjunction of single whole chromosomes rather than through fragmentation; and that hybridization was an important factor in the perpetuation of the new chromosome number. If transverse division is to be taken as the explanation of the origin of some Nicotiana chromosome numbers, one may explain the 10 haploid chromosomes of certain species as arising by the occasional transverse division of two long U-shaped chromosomes, and the 12 chromosome type by the transverse division of four long U-shaped chromosomes of certain 8 chromosome species. It is to be noted, however, that the 12 chromosome species have a large number of U-shaped and very long chromosomes.

WINGE's (1917) theoretical interpretation of the origin of new forms by the reduplication of all the chromosomes of two species after hybridization is probably important in connection with Nicotiana species (CLAUSEN and GOODSPEED 1925). The morphological appearance of certain related Nicotiana species indicates that they have a common origin. *Rustica* and *paniculata* differ noticeably only in respect to corolla length and shape, for example. *Nudicaulis* and *trigonophylla* are also similar to each other, and also show the same chromosome relation, 24:12. The three species *glutinosa*, *tomentosa*, and *Rusbyi* show a gradation in the morphological characteristics of the two sections *Tabacum* and *Rustica*, *glutinosa* showing largely *Rustica* characters, *Rusbyi* largely *Tabacum* characters, with *tomentosa* standing in between. Such instances point to hybridization as a possible origination of species.

The possibility that the species have originated as multiples from a type having the basic number 4, might be indicated if the reduction division of any of the hybrids showed this number of bivalents among the univalents or *vice versa*, as has been found in Triticum (SAX, K., and SAX, H., 1924); but no such cytological situation has been found.

SUMMARY

1. Nineteen species of Nicotiana have been studied cytologically, and species with the following haploid chromosome numbers were found: 8, 10, 12, 16, 24. All of these numbers have occurred among the species belonging to the Petunioides section. Among the species of the *Rustica* section, the haploid numbers found were 8, 12, and 24. Differences in shape and size were found among the chromosomes of the different species and in certain cases among the chromosmes of a single species. Counts made in the root-tips confirmed the numbers found in the pollen-mother cells. There are great differences in size and shape between the chromosomes of the somatic cells and those of the pollen-mother cells, the first being very long, entwined, and generally U-shaped, while the latter spherical or bean-shaped.

2. Fifteen species were crossed with the view of discovering if any parallelism exists between their taxonomic relationship and their ability to hybridize. Four degrees of compatibility between the species were distinguished according to the results obtained in the hybridization experiments: (1) Crosses in which the flowers pollinated dropped after the pollination; (2) those in which capsules were produced without seeds, or with seeds which did not germinate; (3) those in which the seedlings died before reaching maturity, and finally (4) those which produced mature hybrids. It was found further that the species with 8 haploid chromosome numbers produced fertile hybrids (as did species with 10 haploid chromosomes); but the ability to hybridize was very low between the species with 12 chromosomes, though less difficult between the species with 24 chromosomes. In crosses between species differing in their chromosome number, success was rare when species with a lower chromosome number were used as female; and in the cases where the cross succeeded in both directions, the percentage of successes was less when the female parent had the lower chromosome number.

3. A morphological description of the species hybrids obtained is presented. In crosses between species with the same chromosome number, the fertile hybrids, generally speaking, were intermediate, but

the great variability of the F_2 plants and the obvious Mendelization of the striking qualitative differences leads one to believe that the genes are distributed as in ordinary varietal crosses. In hybrids between species differing in their chromosome number, the parental type with the greater number of chromosomes was better represented morphologically.

4. In nine of the species hybrids, the reduction division in the pollen-mother cells was studied. Four types of division were found. In *N. alata*×*Langsdorffii* (8×8), the reduction was normal, no univalents being found. In *N. longiflora*×*Sanderae* (10×8), *N. Tabacum Cuba*×*alata* (24×8), *N. Tabacum* white×*Sanderae* Cl_{10} (24×8), and *N. rustica*×*paniculata* (24×12), the number of bivalent chromosomes was equal to the haploid chromosome number of the parent with the lower chromosome number. In *N. rustica*×*N. Tabacum* white tobacco (24×24) a few loose pairs appeared to be present. In *N. paniculata*×*Langsdorffii* (12×8), *N. suaveolens*×*glutinosa* (16×12), and *N. suaveolens*×*Bigelovii* (16×24), no pairing of chromosomes occurred.

In cases in which a regular pairing of chromosomes was observed, the univalents were usually distributed to both poles without splitting during the heterotypic division, though occasionally lagging chromosomes were found. In cases where no pairing was observed, the chromosomes were distributed irregularly to the poles; and in some few cases, particularly in hybrids between species with high chromosome numbers the occurrence of three spindles during the second metaphase was observed. The reduction division of those hybrids in which some few loose pairs occurred during the heterotypic metaphase or before, was most complicated and irregular; and this type of irregularity was observed in a hybrid where the total number of chromosomes was high (24×24); here diads were observed among the tetrads.

The second division was always a regular equational division.

5. The bearing of these results on certain theoretical problems is discussed. Where the reduction division is normal, inheritance appears to be normal. Where the number of chromosomes contributed by the two parents is identical and the reduction division is abnormal, the hybrids show an intermixture of the characteristics of the two parents, presumably because of lack of dominance or because an approximately equal number of dominants is contributed by each parent. Where the number of chromosomes contributed by the two parents is unequal, the hybrids tended to resemble the parent with the higher chromosome number, with but few exceptions. In these exceptional cases, perhaps the con-

tribution of a few striking characters by the species having the lower chromosome number tends to distort the result.

6. The hybrids which reached maturity were completely sterile, with the exception of *N. rustica*×*paniculata*; and this hybrid was partially sterile. Non-viability of the gametes seems to parallel irregular reduction division.

7. The compatibility of the species shows a greater relation to the chromosome number than to the taxonomic status, but the mere fact that two species have the same chromosome numbers does not mean that they will cross. In general a cross can be made more easily when the species with the higher chromosome number is used as the female parent.

8. There is no particular correlation between the vigor of the hybrid and the mode of the reduction, except that in cases where the reduction division is normal, the plants are never weak. Cases were found in which the hybrid was weak although chromosome pairing occurred, as in *N. Tabacum*×*alata*. On the other hand, in *N. Bigelovii*×*suaveolens*, where no chromosome pairing occurred, the hybrid was very vigorous.

9. Weak hybrids can sometimes be aided in their development by a change in external conditions, such as an increase in the relative amount of light.

10. Though the Nicotiana species probably did not originate by multiplication of 4 chromosomes, it is possible that they did originate from one or more 8-chromosome species by a reduplication of certain chromosomes or of whole sets of chromosomes.

After this paper was completed, there was an opportunity for studying cytologically the following two species of the genus:-

N. caudigera Ph. The seeds were collected in Chile in 1925 by Doctor Johnson of the Gray Herbarium. The plants raised are large, branched, with long petioled leaves, cordate below and lanceolate above, having spine-like glandular hairs on the surface and on the margin. The corolla is white with broad cylindrical tube and short, shallow rounded lobes on the limb. In the pollen-mother cells, stained with acetocarmine, the dumbbell shaped chromosomes are constricted so that their number in some stages appears higher than the real one—x = 12. This was clear on permanent slides where, presumably due to the fixation, the chromosomes were shortened, less closely arranged, and their constriction invisible in the deep hematoxylin stain. The root tip cells contain 24 slender, straight or U-shaped chromosomes

N. solanifolia Wolf–*N. cardiophylla* Ph. The seeds were collected in Chile in 1925 by DOCTOR JOHNSON of the GRAY HERBARIUM. The plants raised developed slowly to a short non-branched stem, with many long petioled, broadly ovate and highly undulate leaves. The corolla resembled that of *N. paniculata*, except for being larger and of cream yellow color. The pollen-mother cells stained with acetocarmine, show 12 chromosomes and the root tip sections on the permanent slides show 24 straight or U-shaped chromosomes. Under the name *N. solanifolia* were raised plants from seeds obtained from a commerical source which were quite different from those described above, and resembled more closely *N. rustica* var. *humilis*, except that the corolla was even smaller, somewhat different in shape and slightly violet-tinged on the tube. Their chromosome number was $x = 24$ or $2x = 48$.

The author gratefully acknowledges his indebtedness to Mr. SIDNEY YARNELL and DOCTOR GREGORY PINCUS for their assistance in preparing the manuscript for publication. Especially he wishes to thank Professor EDWARD M. EAST whose aid and helpful criticism made the work possible.

LITERATURE CITED

BAUR, E., 1911 Einführung in die experimentelle Vererbungslehre. 293 S. Berlin: Borntraeger.

CLAUSEN, R. E., and GOODSPEED, T. H., 1925 Interspecific hybridization in Nicotiana. II. A tetraploid *glutinosa-Tabacum* hybrid, an experimental verification of WINGE's hypothesis. Genetics **10:** 278–284.

COMES, O., 1899 Monographie du genre Nicotiana. 80 pp., 7 *planches*. Naples: Topographie Coöperative.

CORRENS, C., 1909 Vererbungsversuche mit blass (gelb) grünen und buntblättrigen Sippen bei *Mirabilis Jalapa, Urtica pilulifera* und *Lunaria annua*. Zeitschr. indukt. Abstamm. u. Vererb. **1:** 291–329.

DUNAL, M. F., 1852 Solanaeceae, Prodromus systematis Naturalis Regni Vegetabilis. **13/I:** 1–741.

EAST, E. M., 1915 An interpretation of sterility in certain plants. Proc. Amer. Philos. Soc. **54:** 70–72.

1916a Studies on size inheritance in Nicotiana. Genetics **1:** 164–176.

1916b Inheritance in crosses between *Nicotiana Langsdorffii* and *Nicotiana alata*. Genetics **1:** 311–333.

1921 A study of partial sterility in certain hybrids. Genetics **6:** 311–365.

EAST, E. M., and HAYES, H. K., 1912 Heterozygosis in evolution and in plant breeding. U. S Dept. Agric. Bur. Plant Ind. Bull. **243:** 1–58, 8 *plates*.

FOCKE, W. O., 1881 Die Pflanzen-Mischlinge. IV+569 S. Berlin: Borntraeger.

GÄRTNER, C. F. v., 1849 Versuche und Beobachtungen über die Bastarderzeugung im Pflanzenreich. xvi+790 S. Stuttgart: Hering.

GOODSPEED, T. H., 1924 Some chromosome numbers in Nicotiana. Amer. Nat. **58:** 381–382.

1923 A preliminary note on the cytology of Nicotiana species and hybrids. Svensk Bot. Tid. **17:** 472–478.

GOODSPEED, T. H., and CLAUSEN, R. E., 1917 Mendelian factor differences *versus* reaction system contrasts in heredity. Amer. Nat. **51:** 31–46, 92–101.

1917 The nature of the F_1 species hybrids between *Nicotiana sylvestris* and varieties of *Nicotiana Tabacum*. Univ. of Calif. Publ. Botany **5:** 301–322, *plates* 37–48.

1927 Interspecific hybridization in *Nicotiana*. V. Cytological features of two F_1 hybrids made with *Nicotiana Bigelovii* as a parent. Univ. of Calif. Publ. Bot. **11:** 117–125.

Hamsly, W. B., 1905 *Nicotiana Forgetiana*. Bot. Mag. London, 131, Pab. 8006.

HEILBORN, O., 1924 Chromosome numbers and dimensions, species-formation and phylogeny in the genus carex. Hereditas **5:** 129–216, *1 plate*.

ICHIJIMA, K., 1926 Cytological and genetic studies on Fragaria. Genetics **11:** 590–604.

KIHARA, H., 1924 Cytologische und genetische Studien bei wichtigen Getreidearten mit besonderer Rücksicht auf das Verhalten der Chromosomen und die Sterilität in den Bastarden. Mem. Coll. Sc. Kyoto Imp. Univ., Ser. B., **1:** 1–199, 5 *Tafeln*.

KLEBS, G., 1917 Über erbliche Blütenanomalien beim Tabak. Zeitschr. indukt. Abstamm. u. Vererb. **17:** 53–119, *1 Tafel*.

KUWADA, Y. 1919 Die Chromosomenzahl *Zea Mays* L. Jour. Coll. Sc., Imp. Univ. Tokyo **39:** 1–148.

LJUNGDAHL, H., 1922 Zur Zytologie der Gattung Papaver. Svensk. Bot. Tids. **16:** 103–114.

1924 Ueber die Herkunft der in der Meiosis Konjugierenden Chromosomen bei Papaver-Hybriden. Svensk. Bot. Tids. **18:** 279–291.

LOCK, R. H., 1909 A preliminary survey of species crosses in the genus Nicotiana from the Mendelian standpoint. Ann. Roy. Bot. Gar. Peradeniya **4:** 195–227, *plates 18–29*.

LONGLEY, A.E., 1926 Chromosomes and their significance in strawberry classification. Jour. of Agric. Res. **32:** 559–568.

LOTSY, J. P., 1912 Versuche über Artbastarde und Betrachtungen über die Möglichkeit einer Evolution trotz Artbeständigkeit. Zeitschr. indukt. Abstamm. u. Vererb. **8:** 325–332.

MORGAN, T. H., BRIDGES, C. B., and STURTEVANT, A. H., 1925 The genetics of Drosophila. Bibliographia Genetica **2:** 1-262.

RASMUSSON, H. 1921 Beiträge zu einer genetischen Analyse zweier Godetia-Arten und ihrer Bastarde. Hereditas **2:** 143–289, *1 Tafel*.

ROSENBERG, O., 1909 Cytologische und morphologische Studien an *Drosera longifolia*×*rotundifolia*. Kgl. Svensk. Vet. Handl. **43:** 1–64, 4 *Tafeln*.

SACHS-SKALINSKA, M., 1921 Recherches sur les hybrides du Nicotiana. Mém. de l'Inst. de Génétique de l'Ec. Sup. d'Agr. à Varsovie **1:** 47–122, *planches viii–ix*.

SAKAMURA, T., 1918 Kurze Mitteilung über die Chromosomenzahlen und die Verwandtschaftsverhältnisse der Triticum-Arten. Bot. Mag. Tokyo **32:** 151–154.

SAX, K., 1918 The behavior of the chromosomes in fertilization. Genetics **3:** 309–337, *2 plates*.

1922 Sterility of wheat hybrids. II. Chromosome behavior in partially sterile hybrids. Genetics **7:** 513-552 *3 plates*.

SAX, K., and SAX, H. J., 1924 Chromosome behavior in a genus cross. Genetics **9:** 454–464, *1 plate*.

SETCHELL, W. A., 1912 Studies in Nicotiana. I. Univ. of California Publ. Botany **5:** 1–86, *plates 1–28*.

SETCHELL, W. A., GOODSPEED, T. H., and CLAUSEN, R. E., 1922 Inheritance in *Nicotiana Tabacum* I. A report on the results of crossing certain varieties. Univ. of California Publ. Botany **5:** 457–582, *plates* 55–58.

STURTEVANT, A. H., 1920 Genetic studies on *Drosphila simulans*. I. Introduction. Hybrids with *Drosophila melanogaster*. Genetics **5:** 488–500.

1921 Genetic studies on *Drosophila simulans*. II. Sex linked group of genes. Genetics **6:** 43–64.

1921 III. Autosomal genes. General discussion. Genetics **6:** 179–207.

TÄCKHOLM, G., 1922 Zytologische Studien über die Gattung Rosa. Acta Horti Bergiani **7:** 97–381.

TAHARA, M., 1921 Cytologische Studien an einigen Kompositen. Jour. Coll. Sc., Imp. Univ. Tokyo **43**: 11–53, 4 *Tafeln*.

THOMPSON, W. P., 1926 Chromosome behaviour in triploid wheat hybrids. Jour. Genetics **17**: 43–48.

TSCHERMAK, E. V., 1914 Die Verwertung der Bastardierung für phylogenetische Fragen in der Getreidegruppe. Zeitschr. Pflanzenzücht. **2**: 291–312.

WICHURA, M., 1865 Die Bastardbefruchtung im Pflanzenreich, erläutert an den Bastarden der Weiden. 95 S. *2 Tafeln*, Breslau.

WINGE, O., 1917 The chromosomes. Their numbers and general importance. Compt. Rend. Lab Carlsberg **13**: 131–275.

Description of Plates

The drawings except figures 60, 65, 69, were made from single sections with the aid of a camera lucida. All figures in plates I and II were drawn by using the large Zeiss microscope with ocular 10 and oil immersion 1/16. Those of plates III and IV by using compensating ocular 12 instead of 10. As reproduced, all figures have a magnification of 1500 diameters.

Plate 1

Figures 1–15 represent the polar view of the mitotic metaphase of the following *Nicotiana* species:

$2x = 48$

Figure 1.—*N. Tabacum*, var. *macrophylla*.
Figure 2.—*N. rustica*, var. *brasilia*.
Figure 3.—*N. Bigelovii*.
Figure 4.—*N. nudicaulis*.

$2x = 32$

Figure 5.—*N. suaveolens*.

$2x = 24$

Figure 6.—*N. glutinosa*.
Figure 7.—*N. sylvestris*.
Figure 8.—*N. paniculata*.
Figure 9.—*N. glauca*.
Figure 10.—*N. tomentosa*.
Figure 11.—*N. trigonophylla*.
Figure 12.—*N. Palmeri* (?)

$2x = 20$

Figure 13.—*N. plumbaginifolia*.

$2x = 16$

Figure 14.—*N. alata*.
Figure 15.—*N. Langsdorffii*.

CHRISTOFF, M., CYTOLOGICAL STUDIES IN NICOTIANA PLATE 1

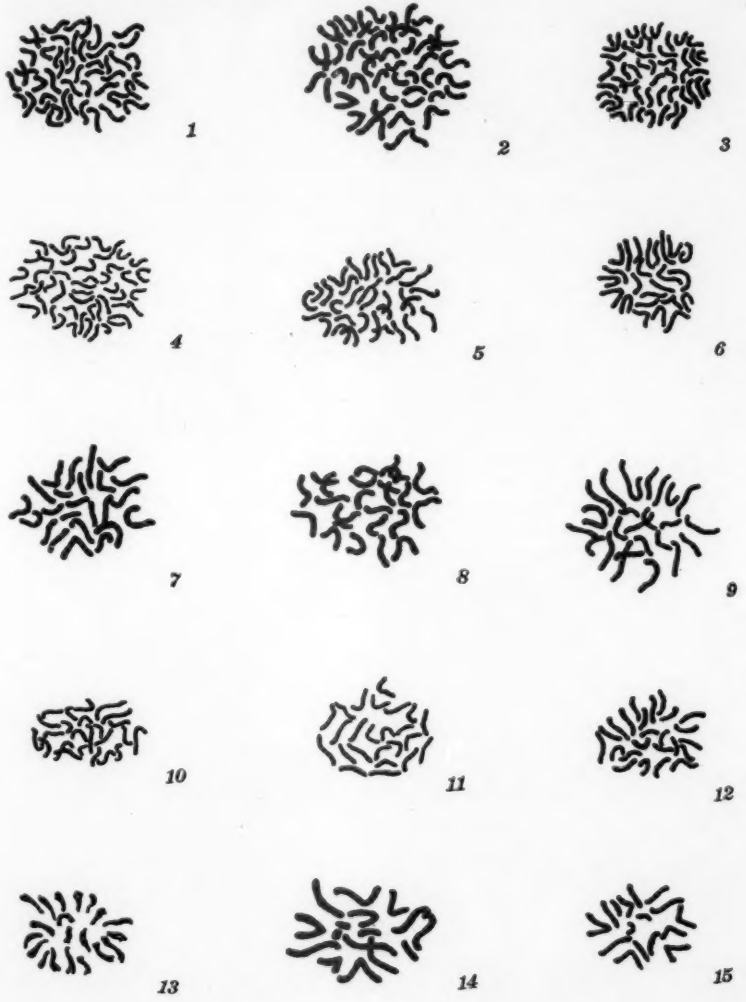


PLATE 2

Figures 16–33 represent the polar view of the heterotypic metaphase of the following Nicotiana species: $x = 24$ Chromosomes

FIGURE 76.—*N. Bigelovii.*
FIGURE 17.—*N. quadrivalvis.*
FIGURE 18.—*N. rustica*, var. *humilis.*
FIGURE 19—*N. Tabacum*, var. *macrophylla.*
FIGURE 20.—*N. viscosa* (?)
FIGURE 21.—*N. nudicaulis.*
$x = 16$ chromosomes
FIGURE 22.—*N. suaveolens.*
$x = 12$ chromosomes
FIGURE 23.—*N. glauca.*
FIGURE 24.—*N. sylvestris.*
FIGURE 25.—*N. paniculata.*
FIGURE 26.—*N. glutinosa.*
FIGURE 27.—*N. acuminata.*
FIGURE 28.—*N. trigonophylla.*
FIGURE 29.—*N. Palmeri* (?)
$x = 10$ chromosomes.
FIGURE 30.—*N. longiflora.*
$x = 8$ chromosomes.
FIGURE 31.—*N. Langsdorffii.*
FIGURE 32.—*N. alata.*
FIGURE 33.—*N. alata.*
FIGURE 34.—*N. alata*—homeotypic metaphase.
FIGURE 35.—*N. alata*—homeotypic metaphase.
FIGURE 36.—*N. alata*—heterotypic metaphase, side view.

CHRISTOFF, M., CYTOLOGICAL STUDIES IN NICOTIANA PLATE 2

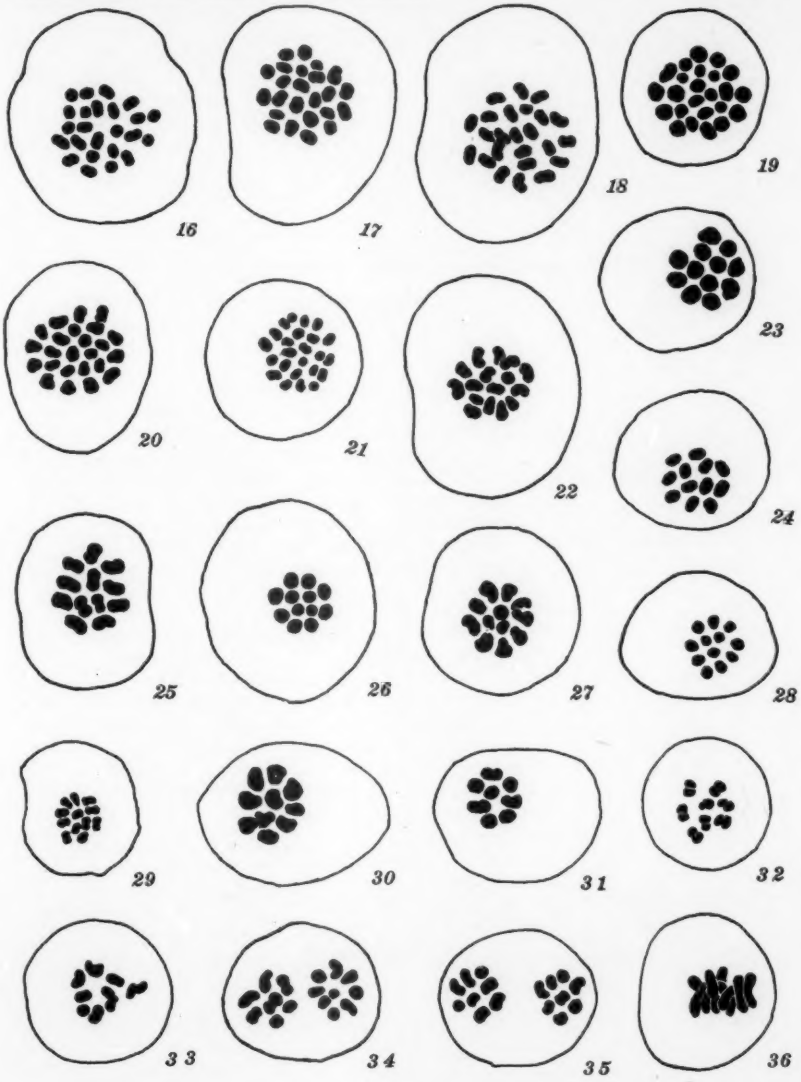


PLATE 3

Different stages in the pollen mother cells of the following *Nicotiana* species hybrids:

N. longiflora ($x=10$)×*Sanderae* ($x=8$)
FIGURE 37.—heterotypic metaphase, side view.
FIGURE 38.—homeotypic metaphase.
FIGURE 39.—homeotypic metaphase.
FIGURE 40.—homeotypic anaphase.
N. Tabacum ($x=24$)×*alata* ($x=8$)
FIGURE 41.—very early heterotypic metaphase, polar view.
FIGURE 42.—very early heterotypic metaphase, side view.
FIGURE 43.—heterotypic metaphase, side view.
FIGURE 44.—late heterotypic metaphase.
FIGURE 45.—homeotypic metaphase, polar view.
FIGURE 46.—homeotypic anaphase, side view.
N. paniculata ($x=12$)×*Langsdorffii* ($x=8$)
FIGURE 47.—heterotypic spindle.
FIGURE 48.—heterotypic anaphase.
FIGURE 49.—homeotypic metaphase, polar view.
FIGURE 50.—homeotypic metaphase, polar view.
FIGURE 51.—homeotypic anaphase, side view.
N. rustica ($x=24$)×*paniculata* ($x=12$)
FIGURE 52.—heterotypic metaphase, polar view.
FIGURE 53.—heterotypic anaphase, side view.
FIGURE 54.—homeotypic metaphase, polar view.
FIGURE 55.—homeotypic metaphase, polar view.
FIGURE 56.—telophase, side view.

CHRISTOFF, M., CYTOLOGICAL STUDIES IN NICOTIANA PLATE 3

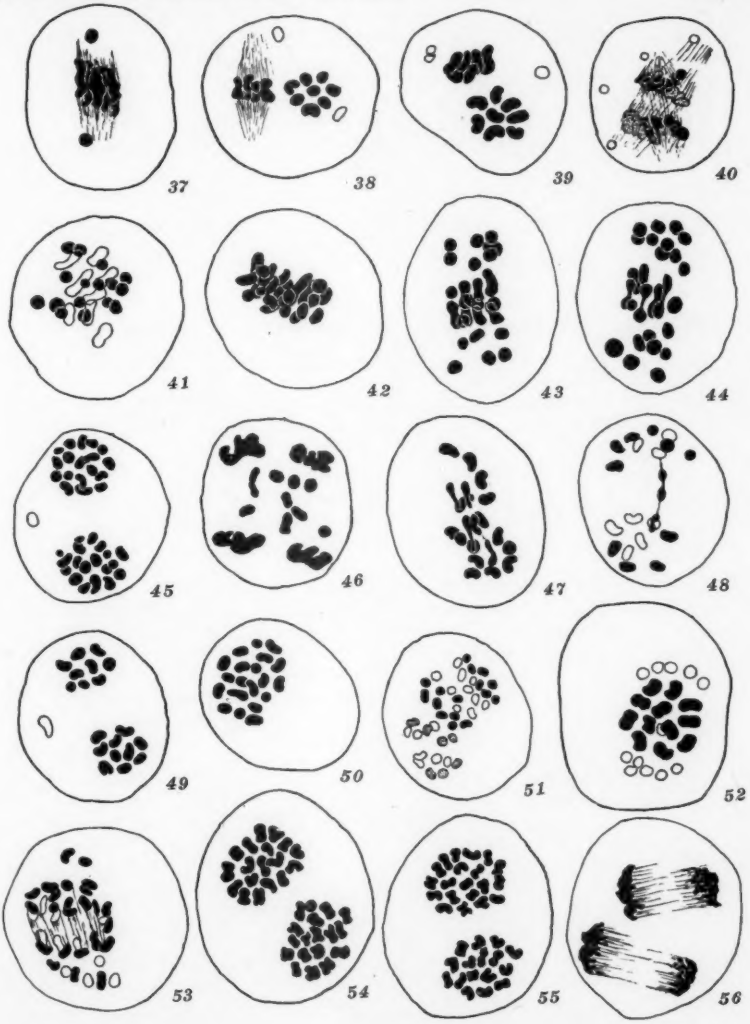


PLATE 4

Different stages in the pollen mother cells of the following *Nicotiana* species hybrids:

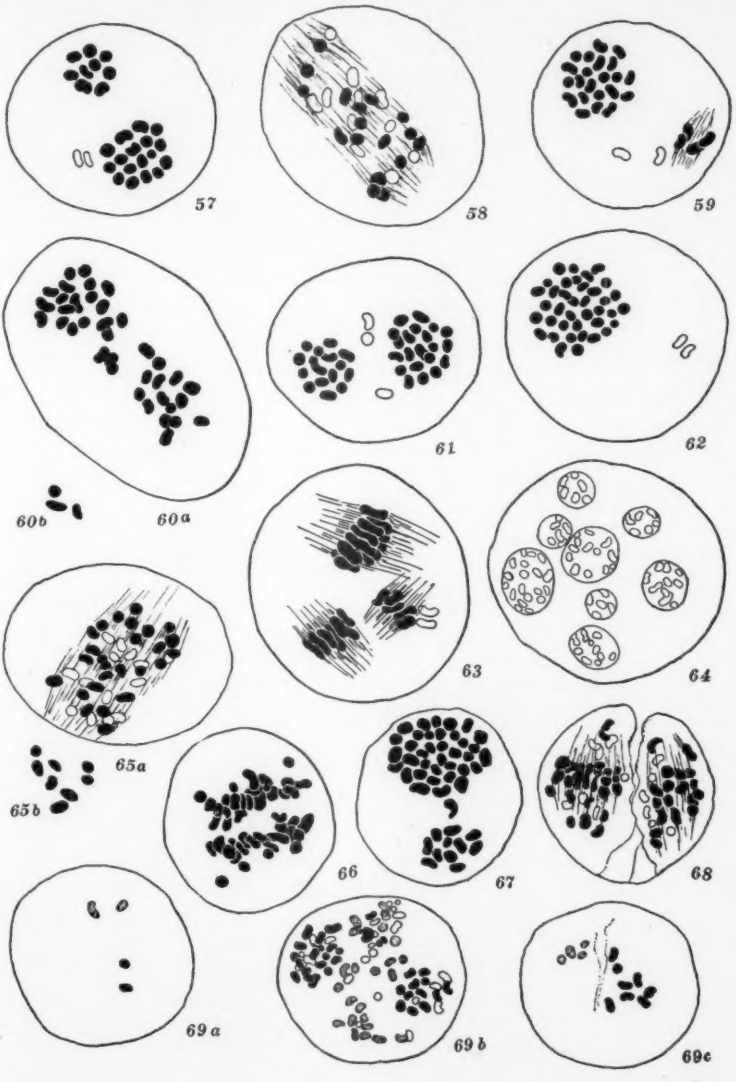
N. suaveolens ($x=16$)×*glutinosa* ($x=12$)

FIGURE 57.—homeotypic metaphase, polar view.
FIGURE 58.—heterotypic metaphase,side view.
FIGURE 59.—homeotypic metaphase.

N. suaveolens ($x=16$)×*Bigelovii* ($x=24$)

FIGURES 60a & b.—heterotypic spindle.
FIGURE 61.—homeotypic metaphase, polar view.
FIGURE 62.—homeotypic metaphase, polar view.
FIGURE 63.—homeotypic spindles.
FIGURE 64.—octad.

N. rustica ($x=24$)×*Tabacum* var. *alba* ($x=24$)

FIGURE 65a & b.—heterotypic spindle.
FIGURE 66.—homeotypic anaphase.
FIGURE 67.—homeotypic metaphase, polar view.
FIGURE 68.—homeotypic metaphase, side view.
FIGURE 69, a, b, c.—homeotypic anaphase, polar view.

CHRISTOFF M., CYTOLOGICAL STUDIES IN NICOTIANA PLATE 4